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MEMOIRS.

On the Structure *of* Hyaline Cartilage.

By G. Thin, M.D. (With Plates I and II.)

Hyaline cartilage is generally believed to consist of a homogeneous ground substance in which are closed cavities harbouring nucleated cells, the opinions of Bubnoff[1] and Heitzmann[2] to the contrary not having hitherto found general acceptance.

The author's views, being founded on results obtained by several methods, some of which are new and others probably not as yet much in use, will be best introduced by a description of these methods and the results obtained by them respectively. The application of caustic potash, being the most important of these, will be considered first. Any one who would apply this process to the study of cartilage should first of all apply it to the cornea of a large animal, such as the ox or the sheep, in order to become conversant with the details of the manipulation and to acquire the necessary faith in its results. For it is on the cornea that in the author's hands it has succeeded with incomparably the greatest frequency. Since first publishing an account of this process[3] the conditions of its success have been somewhat more closely defined, and with the sheep or ox cornea success is now almost invariable. The method by which the author generally operates is as follows:—Fifteen grammes of pure anhydrous caustic potash in stick are ground to powder in a mortar, which if the weather is cold should be previously warmed and well dried; on the powdered potash are quickly poured fifteen cubic centimeters of distilled water, and solution is favoured by stirring. As soon as the potash is dissolved the solution is poured into a narrow glass vessel or porcelain capsule, and the bulb of a thermometer is suspended in the

[1] 'Wien. Sitzb.,' 1868.
[2] 'Wien. Med. Jahrb.,' 1872.
[3] 'Proc. Roy. Soc.,' No. 155, 1874.

upper stratum of the liquid. The heat evolved by the solution of the potash should raise the thermometer above 120° Fahr. When the temperature falls to 107° Fahr. pieces of a cornea are put in one after the other until the temperature falls to 105°. From 107° to 105° is the most favorable temperature. An ox cornea may be cut into four or five portions and allowed to remain in the aqueous humour, which should be saved for the purpose until the solution is ready. Before putting them into the solution the excess of aqueous humour is removed by bringing the portion into momentary contact with the surface of a dry glass slip. After being a few minutes in the solution the portions of cornea are ready for examination. The froth should be removed from the surface of the fluid by a small watch-glass, and a clear drop removed by a glass rod to a glass slip in which a small fragment of one of the pieces is broken up by needles and examined. If the above precautions are carefully observed, it is rare that at least one of the portions is not found to consist of a multitude of the cells described by the author. A solution previously made and heated over a flame has not proved so successful as when the heat produced by the solution of the potash is employed. Fifteen grammes is the smallest quantity that can be relied on as invariably producing sufficient heat. Of course any larger quantity treated with an equal weight of water will be as successful. Ten grammes sometimes, but not invariably, produce sufficient heat. It is necessary that the caustic potash be pure and dry.

A cornea successfully treated should show nothing but the cells. If any other substance of any kind whatever, formed or amorphous, is seen, the operation has been only partially successful. Partial success, in which a greater or less number of cells are seen mixed with other substances, is very common. When a perfectly successful cornea has been obtained it may be kept some time in the solution in a well-stoppered bottle. The smallest particle from any part of it can then be used for demonstration or study when convenient.

On all other tissues except the cornea the operator must at present be prepared to encounter one failure after another, but by persevering not only will all the results be obtained which have been previously described by the author as demonstrable in connective tissue, nerve, and muscle, and those now to be described in cartilage, but probably many others whose discovery awaits a diligent application of the process.

The cartilage covering the head of a frog's femur, or that of a small mammal, is well adapted for treatment by the potash solution. The author has generally cut off the head of the bone and placed it in the solution at the temperature indicated. In a few minutes it falls to the bottom of the vessel, and it can then be removed to a glass slip for examination.

The outer pellicle which represents the cartilage can be easily separated by needles and examined. The substance proper (matrix) of the cartilage can be always seen, but variously modified in different preparations, and some appearances having relation to its arrangements and structure which have not hitherto attracted much attention can frequently be observed; but as these can be better studied by methods shortly to be indicated, it is not necessary to describe them here.

A successful potash preparation shows flattened polygonal cells adhering to each other exactly like an epithelium. In contour, mutual arrangement, and appearance generally they are absolutely indistinguishable from any ordinary epithelial layer. No histologist who had once observed them could hesitate to classify them as a variety of epithelium, especially when the mode of preparation is taken into account. They are seen differently according to the degree in which the potash has acted on the ground-substance. Sheets of cells and more rarely isolated cells are sometimes seen floating free in the liquid. At other times the cells may be seen lying on the surface of considerable fragments of the ground-substance. These fragments sometimes show depressions corresponding in outline, diameter, and relative position with what have been usually described as the cavities or capsules in which the ordinary cartilage-cells are situated, and when the flat cells on them are visible it can be seen that the so-called capsules consist of depressions in the ground-substance, and that the depressions and the more even surface in which these are situated are covered with a continuous epithelial layer. Such a preparation at once shows that the idea of a closed cavity in the ground-substance harbouring a cell has been founded on imperfect methods of observation. Between the free sheets of cells and those seen adhering to the ground substance and its depressions there is an appearance sometimes seen which is intermediate as regards the solvent effect of the solution. The potash may preserve entire a substance which may be described as an interepithelial framework. It follows the lines and angles between the cells, and can be distinguished as differing both from the cells and the ground-

substance properly so called. It is shown imperfectly in Fig. 3.

In the sheets of cells found free parts of them are sometimes seen to be formed by a double layer of cells.

The sheets are often found of considerable size. The cells represented in Fig. 2 formed about the fourth part of a layer which consisted entirely of similar cells to those drawn, and about a sixth part of which consisted of a double layer. It may be presumed, although it is not capable of direct observation, that some of the layers found free in the fluid belong to a superficial layer forming the surface of the cartilage. But the observation of double layers and of fragments of ground-substance which with their depressions are covered with cells shows that they do not all belong to a superficial layer.

The author long searched in vain for the narrow elongated flat cells which he had found in potash preparations of other tissues, but at last succeeded in obtaining a preparation sufficiently distinct to put their existence and arrangement beyond doubt.

Fig. 5 represents rather less than the half of a mass of cells, adherent to the ground-substance, isolated from the head of a frog's femur in May of this year.

The cells are in size and arrangement similar to those which he had previously isolated from the mesentery of the frog and from tendon, by the same process. Their extreme minuteness will be estimated when it is considered that the figure represents as accurately as possible their apparent size when examined by a No. 8 objective and No. 3 ocular of Hartnack's system with the tube full out. The outlines of the nucleus were sharply demarcated, but the cell-substance was represented by a less clearly defined finely granular substance.

Until a more certain method of operating on cartilage by the potash solution is discovered, those who would examine these cells for themselves must possess in a high degree the virtues of patience and perseverance. A strong conviction of the existence of a unity of plan in the general structure of the tissues, and observations on the sclerotic of the frog by maceration in aqueous humour, which will afterwards be described, encouraged the author to continue subjecting cartilage to the action of potash after a number of failures greater than he can recount, and until he had obtained preparations sufficiently distinct to enable him to consider the existence of layers of flat cells, epithelial in their form and

arrangement, in the substance of cartilage as a settled question.

This is not intended to discourage other investigators, who may probably have more frequent success, but to suggest the advisability of the attention of histologists being directed to the study of the conditions of success of a process which produces so important results.

One of the most successful series of preparations was obtained from the head of a frog's femur, which was operated on after the animal had been left dead twenty hours at the temperature of a room in London in the end of May. A number of vertical incisions were made through the cartilage before the head of the bone was put into the solution; but as this was amongst the last experiments that were made, it is at present uncertain whether the time that had elapsed after death and the incisions had anything to do with the success.

The results obtained by subjecting cartilage to the action of nitrate of silver are now to be described.

The author believes them to be mostly, if not entirely, new. The success which has been obtained is due not so much to any special merit in the methods employed as to the number of preparations that were made. The light that was thrown on the structure of cartilage by potash preparations stimulated him to a more persistent application of silver than he would probably have otherwise undertaken. The appearances seen will first be described, and an attempt will afterwards be made to explain them in conjunction with those obtained by other methods.

One of the commonest effects of the action of nitrate of silver on cartilage is a uniform dark-brown staining of the ground-substance. A section so acted on shows a framework of dark substance enclosing in its meshes circular or oval colourless spaces. This appearance is figured by Ranvier in the part that has appeared of his 'Traité technique d'Histologie,' p. 283, and need not, therefore, be repeated here. To the best of my knowledge that is the only effect produced by nitrate of silver that has hitherto been noticed.

The cells on the surface of articular cartilage may be demonstrated in the following manner:—The sections are made from the newly disarticulated head of the frog's femur, one surface of each section consisting of the free surface of the cartilage, and are placed instantly in a half-per-cent. solution of nitrate of silver.

As soon as all the available surface has been so removed, the sections are transferred from the silver solution to a half-

per-cent. solution of common salt and allowed to remain in it only a few seconds. They are then either at once placed in a drop of glycerine on a glass slip, or are put for a few seconds in distilled water, and thence transferred to the glycerine. The glass slip is placed in sun-light. After a quarter to half an hour it will be seen that the free surface of the cartilage is covered with uncolored, generally rectangular, spaces, arranged not unlike the stones that form the pavement of a causeway, and between the spaces are fine lines of a darkly stained substance. The white spaces correspond, as will be seen, to cells. It is further seen that the cells are arranged in quadrangular, triangular, or irregular groups, and that the groups are separated from each other by broader lines of dark substance than those which separate the individual cells.

In the best preparations the widest lines of dark substance do not equal in breadth the diameter of a human red blood-corpuscle, whilst the finer lines between the individual cells are only from one half to a fourth of that breadth. A careful examination will show, in some of the cells, a large round nucleus indicated by a fine unstained ring, which refracts light differently from the substance by which it is surrounded.

A change of focus brings into view the ordinary ground-substance of the cartilage, stained of a brown colour, and distributed in it the ordinary cartilage-cells, more darkly stained, and irregular in outline. A comparison of these with the nucleated quadrangular cells on the surface shows that they differ in size, form, arrangement, and staining capacity.

The surface-cells are seen in Fig. 1. The intercellular substance is exaggerated in the drawing. In the best preparations the epithelial nature of these cells is evident enough, even if it had not been demonstrated by the potash solution.

The knowledge obtained by the use of the potash solution, that layers of cells epithelial in arrangement exist in the substance of cartilage, and the now-established fact that silver solution can demonstrate epithelial layers in the substance of the cornea, although the cornea had been treated for years by silver without these having been seen, rendered it reasonable to persevere in an attempt to obtain similar silver preparations in cartilage, in spite of a long-continued series of negative results.

Thin sections made with a very sharp razor transversely *through the* cartilage covering the condyle of the femur of

the frog and young rabbits (the animals at my disposal) were transferred directly to the silver solution, and then immediately placed in glycerine, and exposed to sun-light. Frogs that have died from disease, and especially frogs and rabbits in which a mechanical or other irritation has been applied for several days to the shaft of the lower third of the bone, are best adapted for its demonstration, but successful preparations can also be obtained from healthy animals.

Fig. 6 represents part of an epithelial surface obtained by this method from a transverse section of the condyle of a frog's femur. In this preparation the extent of surface showing the silver markings (all of it has not been shown) is the largest that was obtained. For a short time after it was prepared the nuclei of some of the cells were distinct. The drawing was made a few days after the preparation was mounted (in May, 1875). Since that time the preparation has considerably deteriorated, but it is still (August 21st) perfectly demonstrative to any one at all familiar with silver preparations. The form and arrangement of the cells will be best understood by an examination of the figure, which represents the preparation with great accuracy.

It is well known that nitrate of silver may produce two distinct results when it permeates a tissue in solution. It may diffuse itself through the ground-substance, which is then stained a varying shade of brown, whilst the interstices which are present in the tissues are unstained, and are then seen as colourless canals and spaces, or it may, while still staining the ground-substance a shade of brown, produce in the system of interspaces, by contact with the lymph-fluid which is present in these channels, an abundant black precipitate of albuminate of silver.

The spaces and canals which in the former case are indicated by their want of colour, are in the latter indicated by the outlines of the black deposit with which they are filled. Both these effects can be produced in hyaline cartilage by nitrate of silver.

To begin with that in which there are white interspaces in a dark ground. The author has found three distinct kinds of effects so produced, which are respectively represented by Figs. 10, 12, and 14. That represented by Fig. 10 will at once recall the white stellate spaces and communicating canals seen in a silver cornea. There is a difference to be noted, due, probably, to the unequal permeability of the tissues, rather than to a difference in their relative structure. In a silver cornea the stellate spaces and lines are found at all depths of the substance acted on. In cartilage

the author has only found them on the surface of sections cut fresh, and then submitted to the action of the silver.

By depressing the focus the uniformly brown ground-substance, with the more darkly coloured cells interspersed, was in such sections invariably brought into view.

There is a certain similarity in the figure to the drawings which have been published by Bubnoff and Heitzmann. Although in strict detail it corresponds with neither, the author considers such preparations as confirmatory of the general principle laid down by these histologists, that there are no closed capsules in cartilage. Heitzmann interprets his preparations as demonstrating that the cartilage-cells communicate by elongated protoplasmic processes. Without at present entering on the question of the existence of such processes, it may be stated that in the author's opinion Heitzmann's drawings show that what he had under his observation was a system of communicating spaces formed by the apposition of two layers being at certain points incomplete.

Another effect of the impregnation of the ground-substance is that shown in Fig. 12.

Such preparations are obtained by removing to an object-glass slip any flat cartilage which is sufficiently thin to be examined entire.

A piece of solid nitrate of silver moistened with distilled water is rubbed efficiently over both surfaces. The cartilage is exposed to the light in glycerine, and when it has become quite dark is freed from the perichondrium under a dissecting lens. (In the frog several distinct layers of large flat polygonal cells, joined like any ordinary epithelium, are found in the perichondrium, and on its surface a thick network of lymphatic capillaries is indicated by a black silver deposit.) When sufficiently freed from its investing membrane the dark ground-work is seen intersected by a network of white lines which branch from a system of wider and nearly straight spaces, such as that represented in the figure. Either of the sternal cartilages or the thin cartilages which form the walls of the larynx of the frog are well suited for treatment by this method. By changing the focus it is seen that the white lines are present at all depths of the cartilage.

The third effect of impregnation of the ground-substance which I have to describe is of a different kind. It is represented in Fig. 14. The condyle of the femur of a kitten a day old was firmly and persistently rubbed over by solid nitrate of silver, which was several times moistened during the opera-

tion. The cartilage was then cut in thin sections more or less perpendicularly to the long axis of the bone, the sections being placed as they were cut in glycerine. They were then exposed to sunlight, and after a few hours examined. In some of the sections the ground-substance was found to be impregnated a dark brown in the form of uniform parallel straight bands or ribbons about the breadth of the diameter of a human red blood-corpuscle, which were separated by white (unstained) lines of a quarter to half the breadth. The alternation of the broader brown and narrower white lines is of the most regular kind. At the spaces which indicate the position of the ordinary cartilage-cells these bands are interrupted.

The part of the cartilage next the bone shows them most frequently. They are found, however, in all parts of the cartilage except the most superficial layers, but this may be accounted for by proximity to the directly cauterized surface. From sections of the condyles of three kittens so treated and examined about a score of available preparations were obtained, and as they are still (after about four months) good it is hoped they may be permanent.

Opportunity was wanting to extend this series of observations to other mammals, but two similar preparations were obtained and preserved from the cartilage of the head of the femur of the frog. As they differ in no respect from those seen in the kitten's cartilage, except in so much as the white lines were relatively broader, no drawing of them has been given. No attempt has been made in the figure to imitate the colour of the brown staining, and the lines are more wavy than they are generally seen. Otherwise the representation is accurate. Only a fraction of a preparation so marked has been drawn, the extent of surface showing this peculiar impregnation being in many of the preparations very much larger than that shown in the figure. The interpretation of this appearance will find its place further on.

In some silver preparations there is a more or less abundant deposit in the substance of the cartilage, and in every instance the form in which the deposited substance is seen may be placed under one of two types. One of these types is shown in Figs. 7 and 8, and the other in Fig. 9. Preparations like that copied in Fig. 8 are obtained either by rubbing over the surface of the cartilage with the solid nitrate or by placing sections in the silver solution. What the conditions are that produce this appearance, and not the others previously described as being sometimes obtained by a similar procedure, have not been determined.

It will be seen from the drawing that the silver is deposited in a connected series of black curves and circles, and when the deposit is not too abundant it can be observed that the ordinary cartilage-cells are mostly found in the area of the deposit. This is still better seen in preparations like that represented in Fig. 7. This drawing represents a section through the cartilage of the knee of a frog that died from disease. The section was placed in a silver solution, and as is shown in the drawing a large part of it was covered with a network of silver deposit. Where the network was complete it was found that the encircled ground-substance was stained a faint brown and that no cells were visible. That the cells were in line with and covered by the deposit was shown by an examination of the edge of the area of deposit where it was incomplete.

At this part the position of the cells was indicated sometimes by a white unstained circle, and sometimes by a similar circle partly filled by the silver precipitate. It is evident, then, that the precipitate takes place most easily in the circular spaces, and afterwards in curved tracts of tolerably uniform breadth which connect the spaces.

A general or unsystematic precipitate of silver in such preparations was never observed, nor was there ever a departure from the usual type either in the size of the meshes of the network or in the breadth of the tracts of which it is composed.

The similarity between this network and that of the lymphatic capillaries which are indicated by silver deposit in the perichondrium is evident.

Metallic deposit in silver preparations is sometimes found in a different relative position. In that part of the articular cartilage which is near the bone the ground-substance has, as is well known, a peculiar arrangement. The cavities or depressions known as capsules appear much larger, and the intermediate substance assumes somewhat the character of a framework, arranged in parallel beams, which are connected by transversely disposed arches. The metallic precipitate at this part of the cartilage is frequently found deposited in the centre of the beams and arches, whilst the contained cellular spaces may be free from it.

In preparations of this kind, in which a small portion of bony substance has been removed in continuity with the cartilage, I have been able to trace the continuity of the tracts of deposit with similar tracts in the bone, a fact of which the significance in relation to the nutrition of cartilage is sufficiently evident. This form of metallic deposit is shown in Fig. 9.

In a paper presented to the Royal Society, an abstract of which is published in the 'Proceedings,' No. 158, 1875, the author has described a number of appearances seen by the application of a method which he thus summarised:—"Transparent animal tissues, when sealed up fresh in aqueous humour or blood-serum by running Brunswick black round the edge of the cover-glass, undergo a series of slow changes, by which, generally within a period of two to five days, anatomical elements, mostly otherwise invisible, become distinct." It was in studying cartilage in the sclerotic of the frog that in the spring of 1874 the author observed for the first time that by this method flat cells could be seen in layers in cartilage. The successful preparations sealed up as described in aqueous humour, and allowed to remain for 24—48 hours (at the temperature of London in June), showed, in addition to a large polygonal epithelium, rows of narrow cells joined to each other end to end, and in contact with other rows on each side. These cells are represented in Fig. 17.

The cells were perfectly hyaline in appearance, with the nucleus well marked. Attempts made this year to obtain similar preparations in the sclerotic of the frog have failed. A series of observations made on sections of the articular cartilage of the carpus of the sheep treated in this way gave the following results. Very thin transverse sections embracing the free surface of the cartilage, show after maceration a distinct layer of rounded cells with a central nucleus. Smaller layers of similar cells may be sometimes seen in very thin transverse sections which do not include the surface of the cartilage, but this appearance is rarely found. In both cases the cells are distinguishable only by the very fine borders which indicate the cell and nucleus, their refractive power differing so little from that of the surrounding medium that it is only when the section is very thin, and with good light and a good lens, that they need be sought for. There is no granular protoplasm in the cell. The ordinary cartilage-cells, much fewer in number and more or less widely separated from each other, can be always observed in such preparations; and as they are in these circumstances seen as well-marked, granular, irregular protoplasmic bodies in the centre of the cellular spaces, it is not possible to confound the two kinds of cells.

The narrow elongated cells seen in the sclerotic and found in the cartilage of the frog's femur, by solution of potash, were not seen in these preparations.

In many of the sheep-cartilage preparations isolated flat cells were found free in the fluid. These were found when

every precaution had been taken to ensure that the sections did not include the free surface of the cartilage. Individual cells so seen are represented in Fig. 16.

In regard to the cells usually seen in cartilage the following is to be added to the methods explained in the text-books of histology:—An ordinary, not necessarily very thin, transverse section is placed on a glass slip, in aqueous humour or blood-serum, and cut in pieces by a sharp knife, an oblique direction being given to the incisions. The pieces are then sealed up in the ordinary way. To increase the chances of success it is advisable to seal up a number of preparations at one time. On the second day or afterwards the obliquely cut edges should be carefully examined. Supposing a preparation to have succeeded, in the oblique edge of the incised surface the same protoplasmic clumps that indicate the cartilage-cells in the rest of the section will be found, but also there may be detected at intervals pear-shaped protoplasmic masses lying free in such of the cellular cavities as have been laid open by the incision. The projecting end of the protoplasm is irregularly round, but the end which is towards the section tapers to a fine prolongation, which is lost in an exceedingly delicate, somewhat glistening fibre, which enters and is lost in the substance of the cartilage. It is easy to identify this branched mass of protoplasm as being of the same nature as the cellular masses which are covered by the cartilage-substance, and to satisfy one's-self that the process has been rendered visible, because the cavity in which it is contained has been laid bare.

This appearance is represented in Fig. 20, the depth of shading in the drawing being, it is to be understood, intended to make more prominent the contours of the different elements rather than to indicate the relative degree of difference of their refractive properties.

In sections so sealed up the substance of the cartilage can sometimes be seen to be composed of parallel bands, having approximatively the diameter of a human red blood-corpuscle. The contour of these bands is well defined, resembling in breadth and arrangement the similar bands that have been described as being sometimes seen in silver preparations. The size and arrangement of these bands are rendered in Fig. 15, which may be compared with Fig. 14. In Fig. 15 the depth of shading is intended to enable the reader to realise more easily the described arrangement. In the preparation the contours are rendered visible only by slight differences of refraction.

In such preparations these bands may be sometimes seen

to be arranged in groups, the divisions between the groups being wider than those between the individual bands. It has not been considered necessary to give a drawing of these groups, which morphologically find their analogues in other forms of connective tissue.

In sealed preparations of cartilage there are sometimes to be seen lying free on the surface of the section, or more frequently free in the fluid near its edge, isolated flattened cylindrical bands, of a like breadth to those described as being arranged in parallel layers in the cartilage-substance, but differing from them when *in situ* in the following particulars:—They are puckered transversely, the puckerings being formed by parallel transverse ridge-like discs separated from each other by a hyaline substance. Frequently the end of such an isolated band bulges over the end of the cylinder as a flocculent coherent mass. Fig. 19 is a copy of a drawing made by Dr. Ewart from a preparation of the head of a frog's femur an hour after it had been sealed in blood-serum, which was obtained by beheading the animal. Such isolated bands can be seen in preparations of other forms of connective tissue similarly treated, and more abundantly if the tissue has been inflamed. When seen isolated it is impossible to distinguish the form of tissue from which they have been separated. The appearance of such an isolated cylindrical body, whether from hyaline cartilage, neurilemma, or an inflamed cornea, is identical. As in some tissues this cylindrical band is farther resolvable into a number of exceedingly fine fibrillæ, the author in his previous histological papers, taking the fibrilla as the ultimate unit, has applied the term primary bundle to such a cylinder or rod.

Now follows the history of a remarkable preparation. In December, 1874, a number of aqueous-humour preparations of transverse sections of the cartilage of the metacarpus of the sheep were sealed. One of these, about which nothing was noted, although it was presumably frequently examined during the first fortnight, was laid aside in order to observe any further changes that might follow. It was not looked at again until July of this year, and when then examined it presented the following microscopic characters:—The substance of the cartilage, to judge from its uneven appearance, had apparently undergone some disintegration; the granular protoplasmic cells had become very small, and lying free in the surrounding fluid were swarms of large, irregularly and variously shaped, finely granular bodies, beset with vacuoles which corresponded in shape and size with

nuclei. It was impossible not to observe the identity of appearance with that of the bodies described as giant cells or myeloplâques. By a careful examination the presence of similar bodies on both surfaces, and in the thickness of the sealed cartilage, could be detected. A drawing of one of the free "giant cells" seen lying close to the edge of the preparation is given in Fig. 18.

The curious part of the history is what follows:—The preparation, with others, was taken from the author's house to Dr. Ewart's room in the museum of University College, and must have remained essentially unchanged there for more than twenty-four hours, as the day after he had received the preparations Dr. Ewart was able to demonstrate the structures in question to Professor Ray Lankester. During the following week the preparation remained in the same room, but was not further examined. It was then brought back to the author, and again examined by Dr. Ewart and himself. To their astonishment every trace of the giant cells had disappeared. The fluid contained threads of streaky looking protoplasmic substance, and swarmed with bacteria, in active motion, in the forms known as coccos, rods, and leptothrix.

The development of bacteria in the preparation took place whilst it was in University College. This, be it remembered, was in the end of July, the preparation having been sealed in the middle of December.

It is well to add that the section of cartilage was from a full-grown sheep, and quite clear of the bone.

One other preparation remains to be described. A pin was pushed through the knee-joint of a frog from side to side between the femur and tibia, the cartilage not being injured further than would result from the presence of the smooth foreign body in the joint. After seven days the frog was killed, and the condyle of the femur placed for three days in a quarter-per-cent. solution of osmic acid. Transverse sections were then made and mounted in glycerine. Thin sections only were sufficiently transparent. The colouring was found to be unequally distributed. Broad winding tracts, darkly stained, contrasted with comparatively faintly stained intermediate portions, and what was especially noteworthy, the coloured tracts contained the ordinary cartilage-cells. The intermediate faintly stained, homogeneous-looking substance contained no visible cells, and indicated by an appearance of splitting the described division of cartilage into polygonal areas.

The deeply stained cellular areas and lightly stained in-

termediate portions are represented in Fig. 13, but the transition between the two is more abrupt in the plate than it is in the preparations.

An attempt will now be made to construct a theory of the structure of hyaline cartilage by which the above data will receive a sufficient explanation.

In regard to the appearances described, as seen by treatment with potash and by maceration in sealed aqueous humour or serum, the facts being once admitted, there can be little difference of opinion as to their interpretation. With regard to the nitrate of silver appearances, there may not be so much unanimity. It may, however, be taken for granted, without further discussion, that the dark lines represented in Fig. 6 indicate the dark lines of epithelial (endothelial) cells. In regard to the uncoloured spaces represented in Figs. 10, 12, and 14, there is room for discussion. Fortunately the same appearances are found in silver preparations of tissues, in regard to whose structure more definite information is obtainable. For example, let the eye of a frog be placed entire in a half-per-cent. solution of nitrate of silver, after a minute or two let the anterior corneal epithelium be romoved, and the eye replaced in the solution for some minutes longer, and then finally removed. The cornea is then to be excised, placed for a second in half-per-cent. salt solution, and then exposed to the light in glycerine. After it has become brown let the upper layer of the cornea be removed at some parts under a dissecting-lens. The following fact is then frequently to be made out:—the silver has diffused itself equally through the whole thickness of the more superficial layer, but has not penetrated to the deeper layer.

There has been evidently an obstacle to the penetration of the solution from the upper to the subjacent layer. The nature of the part or whole of the structure which constitutes this boundary or obstacle may be learned by a rare chance in the frog's cornea itself, but may be more easily shown in the cornea of the mouse. The mouse's cornea is peculiarly well adapted for the demonstration of many points in regard to the histology of that structure. If a mouse's cornea be excised by a single stroke of the scissors and placed in silver solution, and be thence transferred to the light in glycerine, the epithelium of Schweigger-Seidel, of the existence of which it has taken so long to convince some histologists, may be demonstrated with comparative ease.

It is not meant that success in its demonstration is invariable in the cornea of the mouse, but it succeeds in that animal as frequently as many of the ordinary histological processes

which are not supposed to be attended with any special difficulty. The layer of larger flat cells indicated by the silver lines will be found on disintegrating the tissue by needles to correspond to the surface which limits the penetration of the silver in the instance above indicated. It may be taken, then, as a fact that a silver solution may permeate through a tissue, but be arrested by an epithelial layer in the depth or thickness of the tissue. To associate the narrow and anastomosing colourless linear spaces that permeate the substance of a silver cornea with the cells of a similar nature seems justified when it is considered that by potash solution there can be isolated from the cornea multitudes of elongated, narrow cells, and that in gold preparations of the mouse's cornea, and more rarely in the cornea of the larger mammals, rows of similar elongated cells can be traced, taking their departure from layers of polygonal flat cells, and penetrating the interstices of the tissue.

In the mouse's cornea treated by gold the layers of flat cells capable of demonstration are, in the author's experience, composed of smaller cells than the layers of larger cells which are seen in silver preparations.

In potash preparations cells corresponding to those of both layers are isolable. The smaller cells can be seen occasionally in a healthy mouse's cornea treated by gold, but more readily when the structure has been inflamed for a few hours before the animal has been killed.

In the cornea of a frog sealed up in aqueous humour the author, in conjunction with Dr. Ewart, saw the elongated narrow cells *in situ* covering the primary bundles, applied, therefore, to structures having a signification similar to the darkly-stained bands in the silver-treated cartilage (see Figs. 14 and 15). The unstained lines in the cartilage preparations correspond to the rows of cells seen by them in the cornea. When it is considered that elongated narrow cells, similar to but shorter than most of those which are seen in the cornea, exist in cartilage, the presumption becomes very strong that in the cartilage, as in the cornea, they invest the narrow bands of tissue on which they lie, and that the unstained lines seen in section correspond to the cellular investment of the darkly stained cylinders of ground-substance into which the silver solution has permeated.

Recklinghausen's doctrine of *Saft-kanälchen* rests mainly on the fact that in silver preparations communicating colourless spaces are seen in a dark ground, and the unstained stellate spaces in the cornea have been regarded as peculiarly well adapted for its demonstration. Recklinghausen, believing

that the fibrillary tissue is knit together by a cement-substance, has taught that this cement-substance of the cornea is tunneled by a system of communicating canals, and that it is this system of canals which is not stained by silver.

The author dissents from this opinion, believing that the demonstration of layers of flat cells covering free spaces in the cornea, and of the stellate cells as being situated in spaces formed by the separation of these layers from each other at given points warrants the conclusion that Recklinghausen's *Saft-kanälchen* are not closed canals, but are spaces left by apposed membranes receding from each other. In narrower spaces thus left the branches of the stellate cells ramify. Recklinghausen confounded these spaces between the layers (*Saft-kanälchen*) with Bowman's corneal tubes, which the author subsequently confounded[1] with the lymphatic channels in which the nerves lie.

The corneal tubes described by Bowman are different both from the so-called *Saft-kanälchen* of Recklinghausen and from the lymph-channels of the nerves. They are spaces between the primary bundles of the cornea, and have never been described or figured as white spaces in a silvered cornea. In Schweigger-Seidel's plates the injected mass is seen penetrating these straight spaces or corneal tubes, and the author has seen them indicated by rows of fine black granules in silver preparations.

Recklinghausen's *Saft-kanälchen* and Bowman's corneal tubes are neither of them closed channels. The *Saft-kanälchen* are spaces between the laminæ or larger bundles, and the corneal tubes are the spaces between the primary bundles of fibrillary tissue of which the larger bundles are composed. It is evident that the size of the spaces may vary in accordance with the quantity of lymph fluid that passes along the channels, an increase of the calibre of these latter involving no other change than an increase in the extent to which the layers and bundles are separated from each other.

This doctrine, in its full integrity, is now—based on the facts detailed above—sought to be applied to hyaline cartilage, in which the author's preparations show that both the stellate and the parallel systems of lymph-channels exist. The parallel system—corresponding to Bowman's tubes in the cornea—has been shown by an application of the silver method which has not yet succeeded for the cornea, the production, namely, of straight colourless spaces between darkly stained primary bundles.

In interpreting the significance of the epithelial layers

[1] 'Lancet,' Feb. 14, 1874.

found free in potash and seen in sections the following facts are to be borne in mind:—In silver preparations of the surface of articular cartilage a layer of cells can be demonstrated in which the intermediate dark substance is so scant as not only to suggest but to justify their classification as epithelial. In potash preparations layers of cells are found free which, making due allowance for the difference in the mode of preparation, can be identified with a probability which amounts almost to certainty as being similar cells to those which are seen in the silver preparations. The difference between these cells, when demonstrated by silver, and ordinary epithelium similarly treated, the author attributes, not to a difference in the cells themselves, but to their relation to the cartilage-substance, on the surface of which they may be considered to be firmly imbedded. Set free by potash, no difference can be observed between them and ordinary epithelium.

In regard to the epithelial layers seen by silver in sections it is to be considered that in potash preparations layers are seen presenting two kinds of appearance. They are either free in perfectly flat sheets, or they are seen applied to the convex prominences and concave depressions of fragments of the cartilage-substance. It is impossible to be certain that the free sheets in the one case are not the same cells seen still undetached in the other. The analogy of the cornea would seem to suggest that they are not the same.

In the cornea the layers of polygonal flat cells are of two kinds. One kind is most easily demonstrated by silver, and consists of flat layers composed of large cells; another kind is most easily seen by gold, and, although in fortunate circumstances demonstrable as continuous broad layers, is most often seen partially as forming a broad network of small polygonal cells following the channels formed by the separation of the lamellæ in the position of the stellate cells. The former suggests the idea of a thin fascia investing the larger portions of the cornea-substance, the latter of layers of cells investing its larger bundles. Similarly it is suggested that the layers of flat cartilage-cells found free in the potash solution, and those whose outlines and nuclei are represented in Fig. 6, as they were seen in a silver preparation, belong to a thin layer of substance of the nature of a fascia, and that the cells seen adhering to the cartilage-substance in potash preparations invest more closely the cartilage-substance proper. The explanation is hypothetical, but it is a hypothesis suggested by facts.

In regard to the elongated narrow cells nothing can be

considered as proved except their existence in cartilage, but analogy with other tissues suggests that they are applied to the surface of the cylindrical bodies seen in silver and serum preparations (primary bundles of the author as previously described in regard to other connective tissues).

In reference to the appearance of a coherent circular mass bulging from the end of a separated portion of such a band or cylinder the following explanation is suggested :—It is highly probable, for reasons which need not now be entered on, that in all the forms of connective tissue these bands or primary bundles are invested by a resistant delicate membrane on which the narrow elongated cells lie. In all tissues in which the author has seen them these bulgings have been occasionally observed, and have been invariably snared at the point of their escape from the cylinder by a sharply defined ring continuous with the contour of the cylinder. These appearances may be well studied in the cornea of a frog which has been broken down by acute inflammation, however produced, which has lasted about a week, the cornea being then stained in gold.

The production of giant cells from healthy cartilage by simple prolonged maceration in aqueous humour in a sealed preparation must be considered, in the present state of pathological inquiry, as a fact of great importance. During the author's experiments with caustic potash, especially in cartilage, he had frequently seen irregular masses occurring in imperfectly successful experiments which resembled in contour, size, and the arrangement of nuclear vacuoles the bodies known as giant cells, whilst at the same time they could be recognised as partially disintegrated single and double layers of epithelial cells and intermediate substance. So accurate was this resemblance that the hypothesis which the author believes to have received complete confirmation from the aqueous-humour preparation had already presented itself to his mind. If giant cells can be isolated from healthy adult cartilage by simple maceration, it is unnecessary to assign any other reason for their presence in the neighbourhood of cartilage and bone which are undergoing absorption during the process of ossification than that, during the disintegration of the absorbed structure, sheets and fragments of sheets of flat (epithelial) cells are set free, but before they are loosened from their connection they have become so much disintegrated that the lines of junction of the individual cells have become obliterated, and nothing remains but a shapeless protoplasmic mass, containing either nuclei still entire or vacuoles corresponding to nuclei that have undergone destruction. The

physiological connection of the structure with the living organism has ceased to exist. The strongest objection that could be raised against this interpretation of "giant cells" —namely, want of proof that layers of flat cells exist—is answered completely as far as cartilage is concerned by the observations recorded in this paper. Kölliker does not bring forward a single argument in support of his hypothesis that giant cells or osteoclasts are actively concerned in the absorption that takes place in growing bone, except that they are found on surfaces in which absorption is taking place, a fact which finds a much simpler interpretation on the hypothesis that they are a product of the absorption. That they are found in ossification that is taking place where there is no cartilage to absorb does not materially affect the argument when it is admitted that they exist in cartilage. Hitherto layers of flat cells have been as little suspected to exist in cartilage as in bone. But even in bone the author relies on more than mere analogy in believing them to exist. He succeeded on one occasion in isolating by potash from the shaft of a frog's femur great numbers of oval cells, and on several occasions he has seen on fragments of bone treated by the solution numbers of round nuclei, the distribution of which corresponded with the supposition that they belonged to cells whose outlines were imperfectly visible, but which in so far as they could be seen suggested an epithelial arrangement.

In regard to the "giant cells" found in pathological processes it is highly probable, in the light of the above observations, that they are simply fragments of altered and disintegrated membranes, covered with flat cells such as are present in all connective tissues.

If the author may be allowed with diffidence to express an opinion in regard to an appearance that has been studied by so many eminent observers, he would suggest that many of the giant cells (osteoclasts) found in the marrow of young bones present when examined under the microscope (no regard being had to any theory) the characters of effete and disintegrating tissue.

It remains to consider what relation the structures above described have to the spaces known as cartilage "capsules" and their contained cells. As has been already stated, fragments of cartilage can be isolated from a mass treated by the potash solution in which a series of concave depressions are distributed in perfect analogy with the distribution of the "capsules." A so-called capsule consists of two such concavities being applied to each other in such a way that

a wide space, unoccupied by cartilage-substance, is enclosed by them. The edges of this space are formed by the apposition of the edges of the concavities, but although in close apposition the two layers remain anatomically distinct. In sections treated by silver white tracts connecting the spaces, such as are represented in Figs. 10 and 11, or as have been shown by Heitzmann, can be obtained, and are indicative of the continuity of the cell-covered membrane that forms the free surface of the concavities and the intervening flat surface. As can be easily shown for the cornea, so is it equally true but more difficult to show for cartilage, that the cell-spaces are nothing more than the separation from each other of distinct laminæ. Positive evidence of this can be found in potash preparations, but it is also supported by silver preparations, and by the result of such a treatment by osmic acid as is represented in Fig. 13. A white space in a brown ground, as seen in silver preparations of connective tissue, depends upon the fact that the thickness of a space from one epithelium (endothelium) to the other of the two membranes whose separation produces it is sufficient to prevent the dark ground-substance coming into focus. Where the cell-covered membranes are in closer apposition it is impossible to obtain a focus that does not include the dark ground-substance that is above or below the layer of cells. Consequently the unstained thin layer is optically lost in the stained tissue. No tissue is better suited to work this out practically than a well-silvered cornea studied under a good immersion-lens.

The ordinary granular protoplasmic cells of hyaline cartilage are analogous, according to the views of the author, to the stellate cells of the cornea and connective tissue generally. In osmic-acid preparations, and in sections soaked in solution of logwood and alum and subsequently treated by acetic acid, when the protoplasma of the cell had shrunk round the nucleus in the centre of the space, he has seen fine glistening fibres enter the cartilage-substance, into which, however, he has not been able to follow them. Such preparations have not been drawn, because it is believed that preparations of a more demonstrative nature may yet be obtained, and because none of them have been so conclusive as the results of maceration in blood-serum shown in Fig. 20. That only one process has been traced for each cell in such a preparation is due to a law that seems to hold good for stellate cells in other tissues, and especially in those of bone, that although there are many fibres given off the fibres which are continuous with the long axis of the cell are not unfrequently, more developed than those which spring from its

sides. The terminal fibres at opposite poles are accordingly often visible when the others are not seen. In the present case one of the terminal fibres is necessarily cut off when the section is made. It is worthy of remark that although when covered by the cartilage the cells in question show no special disposition to assume an elongated form, they invariably do so when the cavity has been laid open.

The drawings of Bubnoff and Heitzmann the author believes to represent spaces that exist between the layers of cartilage. He is thus in accord with Bubnoff more than with Heitzmann. Heitzmann believes that the appearances which he has reproduced are those of a cell and its protoplasmic processes. The author interprets the appearances shown in Heitzmann's own drawings as representing stellate spaces, and sees nothing in them that he can interpret as cell-processes; being thus at one with him as regards the fact observed, but differing from him in regard to its interpretation.

In that part of cartilage which adjoins the demarcating line characterised as the zone of absorption when bone is being formed there is a preliminary absorption which extends further into the cartilage than is generally supposed, although, not being permeable to blood-vessels, it is not so evident. For some distance into the cartilage the separation of the layers which compose the walls of the spaces increases in extent until the intervening narrow communication between two spaces is swamped, and what was originally two spaces with their intervening communication becomes one large space. A section through cartilage at this point, if stained in carmine or picrocarminate, shows the nucleus of each of the cells which lay in the original spaces lying at opposite ends of the newly formed large space, an appearance which without more ado has been deemed conclusive proof of the division of one cell into two. A further separation of the laminæ permits the entrance of new blood-vessels into the space, and in the language usually employed the "capsule" or "cell" is said to be "opened."

In this series of changes the relation of cause and effect between the two processes of increased entrance of lymph-fluid and thinning of the walls of the laminæ has not been determined by direct observation.

The author acknowledges his obligation to Dr. Ewart for executing the following drawings, and other valuable assistance rendered him whilst engaged in the studies which have served as a basis for this paper.

August, 1875.

On a Polystomatous Condition *of the* Hydranths *of* Cordylophora lacustris. By Hugh Price, Demy of Magdalen College, Oxford.

Specimens of the beautiful hydroid polyp Cordylophora were brought by me last summer from the Victoria Docks for the use of the class of practical zoology at University College, London. Professor Ray Lankester drew my attention to certain abnormal-looking hydranths amongst these specimens which appeared to possess a number of supernumerary oral cones. I isolated some of these abnormal specimens and made them the subject of further study.

It should be stated that these observations were made at the end of June, 1875, on specimens which had apparently already produced their crop of gonophores, and consisted almost entirely of nutrient hydranths which were developing with some vigour, as evidenced by the clean and pale flesh-coloured appearance of their supporting stalks. I presume that a crop of gonophores had been already produced by these specimens, since others obtained from the same locality were in full sexual maturity. An outline sketch of two of the polystomatous hydranths is given in Figs. 1 and 2, where *o c* indicate the oral cones. Fig. 1 is a pentastomate, fig. 2 a tristomate hydranth, each cone having an oral aperture at its apex. The oral cones are larger in the latter than in the former, and are seen to present scattered tentacles on their surfaces, in addition to those which are present on the common body uniting the three cones.

The questions which presented themselves for solution in connection with this hitherto unrecorded mode of growth in Cordylophora were—1. Is such a production of supernumerary oral cones a normal phenomenon in this genus? 2. Do the cones proceed to develop into complete hydranths and become divaricated by the production of interposed hydrocaulin? or do they become detached as fissiparous buds? or does the monstrous condition maintain itself permanently? 3. Have the supernumerary cones developed in consequence of an injury to the parent hydranth? or were they indicated already in the earliest condition of the hydranth which bears them?

These questions I have not been able satisfactorily to resolve, but think it desirable nevertheless to place the facts observed on record as well as some observations on artificially produced polystomatous hydranths of Cordylophora,

which may tend to explain the facts observed in the naturally produced specimens of the same kind.

As to the first question, it would appear from the fact that Cordylophora has been attentively studied by two excellent observers, Prof. Allman and Prof. Eilhard Schultze,

Fig. 1.

Fig. 2.

who have not recorded the existence of polystomatous hydranths, that their occurrence must be exceptional. On the other hand, they were not uncommon on a particular mass of the Cordylophora colony, though they were not found on all colonies gathered with this mass—and have not been previously seen by Prof. Lankester in specimens taken at the same season from the same locality.

As to the second question, I endeavoured to obtain a definite answer. I isolated some fragments of the colony bearing each a single polystomatous hydranth. Unfortunately I was not able to follow these specimens with sufficient care, but it appeared after a few days that the polystomatous hydranths had disappeared, not only in the isolated specimens, but in those still living in the general aquarium containing the mass of the specimens. From this it may be concluded that the polystomatous hydranths had proceeded to develop in some way or other. The opinion that the supernumerary oral cones are but a transitory stage in an exceptional mode of development is borne out by a comparison of Figs. 1 and 2, in the latter of which the cones *are relatively* larger than in Fig. 1, and have, further, a

number of tentacles upon their sides, which is not the case with the specimens drawn in Fig. 1.

Question three is suggested by some observations which I made by snipping with scissors the oral cone of normal hydranths. These observations demonstrate that there is a very remarkable power of repair and what may be called "polarity" in Cordylophora. When a cut is made along the long axis of the hydranth the sides of the separated parts almost immediately come together, and after twenty-four hours both halves of the slit hydranth appear as complete hydranths with oral cone and tentacles (Figs. 3 and 4). If

Fig. 3.

Fig. 4.

the cut is made so long as to extend to the proximal end of the hydranth experimented on, the two portions are in a few days found to have developed each a distinct segment of hydrocaulus surmounted by a complete hydranth.

These facts lend support to the view that the supernumerary oral cones observed in the fresh specimens from Victoria Docks may be due to the injury of the parent hydranth by the attack of some crustacean or to other mechanical injury.

Other experiments which I made do not bear directly on the production of polystomatous hydranths, but serve to illustrate the reproductive power of the tissues of Cordylophora.

Some of the hydranths, with a bit of hydrocaulus, were snipped off from a healthy colony and placed in a vessel

which was kept undisturbed for some days. The cut-off hydranths were found to attach themselves by the cut end of the hydrocaulus to the sides and bottom of the vessel and to give rise to new colonies. Thin cover-glasses were placed on the bottom of the vessel, and to some of these the detached hydranths affixed themselves, thus giving a means of studying the mode of attachment. The cut end by which the animal *appears* to attach itself is seen under the miscroscope to have given rise to a knob-like swelling, consisting of endoderm and ectoderm. But it is not by means of this knob directly that the hydranth becomes affixed. Delicate filaments, continuous with the horny perisarc are seen extending from the neighbourhood of the knob, and these fix the organism to the glass. The knob, on the other hand, proceeds to point in a direction away from the surface of attachment, and gradually develops a bud, which elongates into a complete hydranth, with a rapidly growing stalk or hydrocaulus. Thus two main stems are united at the point of attachment, viz. that of the original hydranth cut off for experiment and that of the new hydranth which has grown out from the cicatrix or knob.

Further (at this time of the year and in these particular specimens) there is a great tendency on the part of the proximal segments of hydrocaulus still attached to the colonies from which hydranths have been cut to produce new hydranths. I have even noticed this on old pieces of hydrocaulus overgrown with Vorticellæ and Algæ. I had occasion to cut a considerable piece off from a main stock, and some of the oldest part of the colony was removed with it. After five days there was developed from the cut end of that portion of the old stalk still attached to the main colony a new hydranth with about half an inch of new stalk attached to it.

These observations on reproduction from the hydrocaulus correspond in the main facts with those of Dalyell and Allman on Tubularia, but the production of a new hydranth-bud from the cicatrix-knob of the radical end of a divided hydrocaulus is not recorded in the case of Tubularia. The facts above noted show that Cordylophora is well adapted for the study of some of the phenomena of artificial reproduction.[1]

[1] I may add to these notes that Cordylophora is by no means difficult to keep alive if provided with a *very* large quantity of water and kept in the dark. Specimens taken in June are still alive (October). These specimens consist of fragments of a colony numbering only some half dozen hydranths. Each fragment has been kept in a covered half-pint jar in a dark cupboard.—E. Ray Lankester.

Further Observations *on a* Peach- *or* Red-coloured Bacterium—*Bacterium rubescens*. By E. Ray Lankester, M.A., F.R.S. (With Plate III.)

The growth varying in colour from peach-blue to a ruby-red which I described in this Journal in 1873 (vol. xiii, page 408, Plates XXII, XXIII) has been under my observation from time to time during the last two years, and I have ascertained a variety of new facts with regard to it which add very much to its interest.

Frequency of Occurrence.—I have had no difficulty in obtaining any quantity of the red growth formed by *B. rubescens*; it is probably familiar to most microscopists who occupy themselves with the lower organisms of fresh waters. Almost any mixture of Algæ and Infusoria which is allowed slowly to putrefy becomes the seat of its development—thick, red-coloured crusts forming on the sides and bottom of the jar in which the putrefaction is proceeding, and resembling the coloured films which form on the sides of a bottle of Burgundy wine.

I have received a particular form of this growth (of which more below) from my friend, Mr. Charles Stewart, F.L.S., of St. Thomas' Hospital, who finds it in large quantities in the macerating tubs which he uses for the preparation of skeletons.

Further, from my friend, Mr. Archer, F.R.S., of Dublin, I have received other samples taken in a pond fifty miles distant from Dublin, which I do not feel any doubt are to be considered as growths of *Bacterium rubescens*. To these also I shall allude further below.

Lastly, I am very much disposed to believe that the pink-coloured Spirillum, described by Dr. Klein in the October number of this Journal for the year 1875, is a spirillar phase of *Bacterium rubescens*. Dr. Klein was good enough to show me his specimens, and I base my opinion on a microscopical examination of the growth and on Mr. Page's statement, given by Dr. Klein, as to the absorption-spectrum, which so far as it was observed (the colour not being very intense) corresponds with that of Bacterio-purpurin—the colouring matter of *B. rubescens*.

Variation in Colour.—The main fact on which I rely for the identification of the various forms and aggregations of plastids, assigned to *Bacterium rubescens* as members of a series or physiological species, is their agreement in colour.

I assume it to be highly improbable that two or more specifically distinct organisms would develope in a jar simultaneously, each tinted by the peculiar substance Bacterio-purpurin. At the same time the hypothesis that the various forms observed do form a series belonging to a protean species is confirmed by the observation of all conceivable intermediate annectent forms.

In my former paper I pointed out that the colour of the films was liable to a certain variation, tending at one time to blue and at another time to red. This variation in tint, which I have now constantly observed, occurs in response to definite variations in the conditions of growth, and is, I believe, satisfactorily explained by the predominance of either a blue or a red element of the complex colouring matter Bacterio-purpurin. A certain brownish tinge is also observable under some conditions, and is, I conceive, to be explained similarly.

The bluer peach tint occurs when the growth is young, and has been especially noticed by me when it occurs in the form of a dense film (the plastids being arranged like a mosaic or "tesselate," as in the mycoderma phase of *Bacterium termo*, or in "catenular" series as seen in Plate XXII, fig. 3, of my former paper), encrusting vegetable matter such as dead twigs of trees. The redder colour is assumed when the growth is more luxuriant, and is especially brilliant when the growth takes on the form of large homogeneous discs (to be described in the present communication). In small specimens of these discs and also in small biscuit-shaped plastids from Mr. Stewart's macerating pan I have seen the colour of the greatest intensity, only to be compared to the deepest magenta dye. Also in the same conditions which furnish these most brilliantly coloured examples are to be noticed others of a decidedly *brownish* tint (Plate III, fig. 4, fig. 20).

As will be mentioned below, the conditions under which the homogeneous discs are developed have not been determined, but their occurrence seems to be due *either* to the exhaustion of nutriment in the jar containing the growth, *or* to the dying down of the green unicellular algæ with which *Bacterium rubescens* is usually *most intimately* associated and upon which it may be dependent for oxygen, and such services as the gonidia of the Lichen render to the hyphal fungus.

Variation in the size of the Plastids.—As was pointed out in my previous paper, the form of the plastid of *Bacterium rubescens* may vary greatly. It may be spherical, biscuit-

shaped, filamentous, or irregular. We shall see now that it may also be discoidal. I pointed out previously that there was considerable variation in the size of the plastids, and gave figures illustrating this. The discoid plastids now to be described, whilst retaining a perfectly homogeneous structure, may attain each to the diameter of $\frac{1}{15000}$th of an inch, whilst the largest biscuit-shaped plastids described in my former paper scarcely exceed the $\frac{1}{3000}$th of an inch in length. Under certain conditions it seems that very small biscuit-shaped plastids (very similar in shape and size to the particular form-species distinguished by Cohn as *Bacterium termo*) are uniformly produced by *Bacterium rubescens*. A group of these is seen in Plate III, fig. 4. The plastids do not exceed the $\frac{1}{60,000}$th inch in length. In this case I am able to point to a special condition of life as connected with this uniform shape and small size. These are the only form of *Bacterium rubescens* occurring in the macerating pans of St. Thomas' Hospital.* They occur in immense quantity, covering the bones with a red film and forming crusts on the side of the vessel. It appears to me that the small size of the plastids and their uniform character may be due to the unconscious process of cultivation and domestication to which the museum curator subjects them in his macerating tub. A uniform temperature is maintained, an immense excess of nutritive matter is continually present of a uniform character (namely, decomposing bones),[2] and the macerating tub once established is kept in operation for many years. Under these circumstances it is not unlikely that a particular "breed" or "race" of the protean *Bact. rubescens* has become established, just as very possibly in other parallel cases (for instance, that of *Saccharomyces cerevisiæ*) races or even species of ferment-organisms have been established by the unconscious operation of mankind. I made some experiments with the view of ascertaining whether by changing the conditions of life of the maceration-breed of *Bact. rubescens* its form or colour could be affected in any way. Some of the red scum from the macerating pan was isolated and placed with distilled water in a test tube. It was thus cut off from further accession of nutriment. It was left undisturbed for three months; at the end of this time no change visible to the naked eye had occurred in the

* The colouring matter, which is very rich, gave the characteristic bands of absorption of Bacterio-purpurin.

[2] It should be very explicitly stated that a large proportion of green unicellular algæ is present with the red Bacterium in the macerating pans, intermixed with it.

red sediment formed by the scum, excepting that its colour was a little more intense. On examination with the microscope, however, the plastids were found to have altogether changed the character of their growth. Instead of keeping down to a small size by repeated transverse fission they had all *increased individually* in size. Some were a little more than double the length of the specimens taken fresh from the macerating tub, and presented two, three, or more brightly red-coloured, highly refracting granules in their substance, similar to those seen in the plastids drawn in fig. 3, Plate III, which do not, however, represent these particular specimens. This change of size and character in the plastids in attendance upon change of environment (removal of excessive nutrition) warrants the inference which I had already drawn from the large range of variation in the forms and mode of aggregation of the plastids of *Bacterium rubescens*—that we have in it (and probably also in the true physiological species of colourless Bacteria, distributed under such form-genera as Micrococcus, Bacterium, Vibrio, &c.) an example of an organism which is highly susceptible of change of form in response to minute changes of life-conditions, and in which such changes of form have full license because *form* has very little importance in relation to the *essential* chemical phenomena which characterise the life of this class of organisms and give them specific limits.

Movements of B. rubescens.—In my former account of this organism I stated that I had not observed active "vital" movement exhibited by the plastids except by the "acicular" form. I am now able to modify this statement very essentially. I have frequently observed active vital movement exactly corresponding to that of *Bacterium termo* and *B. lineola* in the biscuit-shaped or bacterioid form of plastid of *B. rubescens*. The plastids drawn in Fig. 2 formed part of a growth in which nearly all the plastids were of this homogeneous character and exhibited the active darting movement of living Bacteria. On other occasions I have found growths of plastids of the character of those given in Fig. 3, which also exhibited the unmistakable vital movements known and described in other Bacteria.

Further, I have on more than one occasion observed slow but continuous undulating movement in the Leptothrix forms of *B. rubescens*, such as are figured in my former paper (Plate XXIII, figs. 24, 25).

The maceration variety from St. Thomas's Hospital (fig. 4) also exhibits active vital movements.

From recent study of *B. rubescens* and other forms of Bac-

teria I am inclined to think that the former is not, on the whole, a more sluggish form than are the colourless species. In the glœogenous condition all Bacteria are motionless, and it is only the biscuit-shaped form of plastid or still more elongated forms which exhibit "vital" movement.

The question as to the mechanism of locomotion in the Bacteria is one which I have not been able to assist in solving by examination of active *B. rubescens*. *A priori*, I suppose all biologists are inclined to adopt the view that the motile plastid of Schizomycetes is provided with one or two protoplasmic flagella. The evidence in favour of the existence of such flagella is very nearly as convincing as any that could be obtained by the actual inspection of a filament attached to the plastid. I confess that I have not been able to *see* the flagella in any form of Schizomycetes which I have studied for the purpose, but I think there is every reason for admitting the truth of the recent observations of Messrs. Dallinger and Drysdale on this point.

New Transition-forms of Plastids and Aggregates.—It will not be necessary for me to repeat, on the present occasion, the enumeration of the various kinds of plastids presented by *B. rubescens*, and the various modes in which these plastids unite to form aggregates, linear, globose, retiform, and tesselate. I shall, without any preface, draw attention to two interesting forms which I have observed since 1873, referring to my previous paper for a general account of the forms assumed by the species.

Fig. 3, Plate III, represents a series of plastids from a growth in which such forms constituted the greater part of the sample. They may be described in the terminology which I have adopted, as multilocular bacterioid plastids—which occur in this case isolated—and free from a gelatinous matrix. Those to the right hand side are interesting on account of their elongation, furnishing, as they do, a transition-form to the filamentous plastids (leptothrix-form) figured in vol. xiii of this Journal, Plate XXIII, figs. 24, 25.

Fig. 5 in Plate III is a small specimen of a form of *B. rubescens* which is rare, and of very great beauty and interest. I had only met with it on two occasions (one specimen being that here figured), when I received, in September last, from my friend Mr. Archer, of Dublin, a gathering which contained a great quantity of it, and very much larger aggregates of it than that here figured. This form, which consists of strongly marked unilocular bacterioid (biscuit-shaped) plastids (almost like an hour-glass in shape), united with the greatest regu-

larity in rectilinear series, may be described as the *rectilinear tesselate* form of aggregation. *Sarcina*, and more especially *Merismopedia*, furnish very similar aggregates of their constituent plastids. The gathering which I received from Mr. Archer contained sheets of plastids in which the plastids were of this hour-glass shape, and arranged in this same rectilinear manner; but the sheets were of larger size, consisting of as many as twelve or twenty rows. The aggregates of this kind very generally are nearly square plates, and have so symmetrical an aspect as to suggest rather a work of art than a natural growth. Frequently, at the sides of the square plate, a group of four, six, or eight plastids has become detached and fallen away, leaving a square hole or blank in the series. I hesitated for some time about regarding these regularly arranged plates as forms of *B. rubescens*, until the study of Mr. Archer's specimens enabled me to satisfy myself that the plastids are really coloured centrally with the characteristic purple-red pigment of that species, and also furnished additional proof of the connection of this form with the other varieties of *B. rubescens*, in the fact that they occurred in his gathering (as they had in those I first observed) in connection with a great abundance of other varieties assignable to that species, such, for instance, as fig. 16 and fig. 21 of Plate XXIII of my former memoir.

The form of the individual plastids, though very strongly marked, was already familiar to me. Some of this form are drawn in figs. 13 and 26 of my former memoir. In fig. 6, Plate III, I have represented a plastid of the same form, which is interesting as giving a transition between this very regular unilocular form and the multilocular forms. In addition to the central hour-glass-shaped cavity filled with coloured material, a small eccentrically placed granule is seen, eating its way, as it were, into the thick colourless wall of the plastid.

The plastids of the regular tesselate form of aggregation cohere laterally by means, no doubt, of the gelatinous substance which forms a sheath to each of them. One would suppose, from the analogy of tissues and cell-development generally, that such a regular aggregate had been formed by a regular process of cell division *in situ*. I am inclined, however, to believe that this is not the case, and that the tesselate aggregations of all kinds of Bacteria, as well as the retiform (such as that drawn in fig. 19 of my former paper), take their origin by the spontaneous apposition of independently developed plastids, just as the network of *Hydrodictyon* is developed.

Macroplasts or Reproductive Discs.—The form of plastid which I have now to describe is one of altogether special interest, since it seems possible that we have in it a definite reproductive form, whilst the phenomena of division presented by it, resulting in the formation of new colonies of plastids, are so exceptional as to be in themselves a matter of interest from the point of view of the histologist. At the same time, should it be found that the colourless Bacteria develop reproductive discs, or macroplasts of the same character as those of *B. rubescens*, we shall have ascertained a possible source of origin for those excessively minute germs which are so widely distributed, and which so readily develop in putrescible liquids.

In the jar of fresh water in which my *Bacterium rubescens* originally developed the growth was continually kept up by the addition on my part of dead animal matter, such, for instance, as an earthworm or a moth, or a limb of a crayfish. After luxuriating for a time, the red growth would always languish and become reduced to a small area, varying in colour from peach colour to brick red, and this film would lie at the bottom of the vessel beneath the black deposit of carbonised *débris*. When it had assumed this state I found that I could always call forth an abundant crop by the addition of fresh animal matter. In September of 1873 the growth had been allowed to dwindle to its very smallest limits, and I then examined some of the very deeply coloured film which lay at the bottom of the glass jar, and was visible by inspection from below. I found it to consist of a felted mass of green unicellular algæ, desmids, various organic filaments, and a large quantity of glœogenous plastids of *Bacterium rubescens*, some in the condition of sheets free from any excess of gelatinous matrix, and forming very highly coloured expanses. Such mycoderma-like fragments (but of much larger extent) as those drawn in Plate III, fig. 10 and fig. 22, were abundant, agreeing in the cavernous shape of the aggregate or frond with the *Clathrocystis* of Henfrey.[1] The most remarkable appearances, however, were a large number of disc-like bodies, of very various size, deeply impregnated with the red colour, distinguished as Bacterio-purpurin. These bodies varied in size from the dimensions of an ordinary biscuit-shaped plastid of *B. rubescens* to that of a circle with $\frac{1}{150}$th inch diameter. The

[1] I am informed by Professor Ferdinand Cohn that he has referred this form of *B. rubescens* to Henfrey's genus as *Clathrocystis rubeo-persicinus*. He also considers some of the forms of my *B. rubescens* to be the *Monas Okeni* of Ehrenberg.

average diameter of these discs was about the $\frac{1}{7\,0\,0}$th of an inch and less. A series of them is represented in the plate (Plate III), which gives a representation of a possible group of these bodies (figs. 7 to 23), as obtained by teazing such a film as that described. With a power of 600 diameters the disc-like bodies are seen to vary both in colour and structure. Some are of the very deepest ruby red (figs. 14, 23); others are paler, but still of a rich colour (fig. 16); whilst others, again (figs. 19, 20), have a decidedly brownish tint. With the power of 600 diameters (Hartnack's No. 8 objective and No. 4 eye-piece) many of the discs appear homogeneous, devoid of all granulation, and of gelatinous border or capsule. This is the case equally with figs. 16, 17, 18, 19, 20, and 21. On the other hand, with the power named, figs. 12 and 13 appear granular, and fig. 9 is seen to consist of a closely adherent mass of plastids with a gelatinous border. Fig. 15, again, is seen to have a relatively larger amount of gelatinous matter separating its highly coloured loculi (each of which corresponds to a plastid), whilst in fig. 7 we have a form in which the primitive glœogenous sphere is breaking up into secondary spheres, each secondary sphere containing numerous highly coloured loculi.

When a higher power of the microscope is applied to these discoid objects—namely, the No. 10 immersion of Hartnack, with No. 4 eyepiece, giving an amplification of 1100 diameters with the best definition and penetration which I know of at that point of magnifying power—some of the apparently homogeneous discs are resolved, and are found to present a minutely punctate structure. Others resist the test and still appear—as I conclude they are (within the physical limits of the word)—homogeneous.

Such homogeneous discoid plastids are represented in fig. 14, fig. 16, fig. 20, and in the series of smaller bodies to the left of fig. 19. The most minutely punctate forms are seen in figs. 18, 17, and 21. When teased or broken by pressure of the covering glass these discoid bodies give evidence of being composed of a somewhat tough coherent substance, and do not diffuse or break up into granules.

The following is the explanation which I have to offer of these appearances. Under certain conditions of growth, which I have not been able to determine specifically, but which are possibly amongst others those of diminished nutrition—the plastids of *B. rubescens* cease to multiply by transverse division or to form filamentous or other irregularly extended growths. They cease also to form gelatinous envelopes and take on a physiologically homogeneous cha-

racter (indicated by the uniform distribution of the colouring matter) and grow equally at the periphery so as to form small discs. These are represented in fig. 11 when of small size.

This uniform homogeneous growth may proceed until single plastids attain the very large diameter of $\frac{1}{100}$th of an inch or more. This is accompanied frequently by great intensification of the colour, indicating a special physiological activity in the development of Bacterio-purpurin. Not unfrequently, however, the process which leads to the elaboration of Bacterio-purpurin is so modified as to give rise to the brown variety of that pigment. Such discs, on account of their large size, I would call "macroplasts." These macroplasts are certainly of a somewhat permanent character. I have kept one under observation for fourteen days without observing any change in its size or structure.

The conditions which are necessary for this change are not known, but clearly enough they exist, and at one time or another the macroplast falls under their influence and takes on a new development. This may occur either when it is of comparatively small size or when it is of larger growth. The addition of fresh nutrient material to the jar containing the macroplasts led to their ultimate disappearance and their replacement by colonies of plastids of types similar to those previously described. Hence I am inclined to infer that under favorable circumstances as regards nutrition the macroplasts break up into immense numbers of small plastids. The first step in this process is seen in the excessively delicate granulation of such specimens as figs. 17, 18, and 21. In fig. 12 we have a small specimen in which the newly forming plastids have attained larger size and so on through the series—a gelatinous exudation being produced as the process of segregation advances (figs. 13, 9, and 15).

If this be a correct interpretation of the appearances depicted in the plate (Plate III), we have an instance of multiplication of "centres of organization" which does not conform to the type observed in ordinary cell-division, and which is comparable to free cell-formation in a blastema. Each plastid in any growth or aggregation of Schizomycetes may be regarded as a unit or centre of organization—just as much so as a true cell, though the Schizomycetous plastid differs from a cell in not possessing a central mass corresponding to the nucleus. The transverse division of an elongated or filamentous plastid of one of the Schizomycetes is equivalent to an act of cell-division and so far the protoplasm of these organisms conforms to the general mode of

multiplication of units or centres of organization which is observed in other masses of protoplasm.

Just as we find exceptional cases in animal and vegetable cells in which a mass of protoplasm gives rise simultaneously to numerous nuclei, each of which becomes surrounded by a segregated mass of protoplasm and produces a numerous cell-progeny by multicentral segregation, so it appears that in the large discoid macroplasts of *Bacterium rubescens* a formation of innumerable new plastids occur—*not* by a process of progressive division into two, four, eight, &c.—but by a simultaneous multicentral segregation. Such appears to me to be the explanation of the minutely granular structure detected only with the highest powers in such specimens as figs. 17, 18, and 21. The granules indicate so many new units or centres of organization, and each of them takes on independent growth, enlarges and finally becomes separated from its fellows by a gelatinous envelope.

It would be necessary in order to establish this view to watch the steps of the process in one and the same macroplast; and it is clear that during the period of increase in the size of the new units, the whole mass must greatly enlarge. I have not succeeded in actually watching the process of development which I suppose may take place, but I draw attention to the fact that there is on the whole evidence of a proportionately larger size in those macroplasts which are coarsely as compared with those which are finely granular. This fact does not, however, count for very much, since one observes granular macroplasts of very various sizes from the $\frac{1}{3000}$th of an inch upwards.

A second development of the macroplasts took place in the same jar after an interval of six months, during which time the "growth" had been carefully fed up, and it had become difficult to find any specimens of them still remaining. The second development of these bodies occurred after the supply of nutrient matter to the jar had again been discontinued for some weeks.

Specimens of the macroplasts in various stages of segregation were mounted in glycerine, where they preserved their colour for a month or so, but subsequently assumed a pale, dirty green tint. Such specimens were sent by me to various naturalists interested in the study of the Bacteria, whilst my friend, Professor Thiselton Dyer, examined the growth in its living condition in my laboratory at Exeter College.

Supposing the most finely granular macroplasts to be mechanically broken up into their minute constituent granules, we should have a very prolific source of those excess-

sively small "germs" from which Bacteria are supposed to take their origin in experimental infusions.

Reasons for assigning Bacterium rubescens to the Schizomycetes and the Genus Bacterium.—It has been suggested to me by some observers who have examined samples of the growths which I have called *Bacterium rubescens*, that the organism in question is not to be regarded as a colour-bearing species of the group of Schizomycetes, but is rather to be classed with some of the glœogenous forms of the so-called unicellular Algæ, such as Glœocapsa. This view appears to rest upon two facts—firstly, upon the assumed absence of a motile phase of the plastids of our organism; secondly, upon the presence of a strongly developed colouring-matter.

The really important agreements between Bacterium rubescens and the Schizomycetes appear to me to lie in the following facts: that *B. rubescens* is one of the *chlorophyll-free* Protophyta (consequently physiologically similar to the Schizomycetes and Fungi), and that it exhibits the characteristic vegetation forms of the Schizomycetes, namely, spherical, biscuit-shaped, elongated or filamentous plastids, devoid of nucleus, and aggregated either in linear series or in sheet-like and massive fronds by means of a jelly-like matrix.

The assumed absence of a motile phase is an error. Precisely under the same conditions and with the same constancy as is observed in the colourless Schizomycetes, active darting movements of the plastids of *Bacterium rubescens* are observed. It is a mistake to allow the commonly observed active condition of the Schizomycetes, such as *Bacterium lineola* and *B. termo*, to dominate altogether in our conception of the essential features of the Schizomycetes. These species exhibit, quite as commonly and abundantly as *B. rubescens*, quiescent phases and glœogenous aggregations distinguished by Cohn as "zooglœa."

There is very good ground for supposing that the common micrococcus which appears before and with the biscuit-shaped *Bacterium termo* in putrescent infusions, and which forms "rosary-chains" and other growths is a phase of the species, whose active condition is distinguished as *Bacterium termo*; and we have similarly in *B. rubescens*—a micrococcus-phase (the spherical plastids described in my previous communication), and catenular rosary-chain aggregations belonging to a species which in a certain phase of growth produces biscuit-shaped plastids, such as those drawn in Pl. III, figs. 2 and 4. These are undeniably referable to the form-genus Bac-

terium, as limited by Cohn, and often exhibit active, darting movements.

The objection which is founded on the presence of colouring matter in *B. rubescens* is easily met. The pigment of *B. rubescens* contains neither phycocyan nor chlorophyll, and one or both of these colouring-matters is present in all Protophyta and Thallophyta, which are not referable either to the Schizomycetes, Saccharomycetes, or to the so-called Fungi. On the other hand, pigmentary matter similar to Bacteriopurpurin has been observed in other forms recognised as Schizomycetes, and is not uncommon in various groups of Fungi. It seems to me that the presence of a red colouring matter in *B. rubescens* is a fact of no significance whatever, as far as its relationship to other forms is concerned. In considering the question of those relationships we should mentally bleach *B. rubescens*.

Further as to the choice of the generic term *Bacterium* for the designation of our organism, I have this to say. From the known facts as to the mode of occurrence of those *forms* of Schizomycetes which Cohn has classed as Sphærobacteria, Microbacteria, Desmobacteria, and Spirobacteria, and from what I have been led to infer as to the connection of the various forms of plastids in my red growth, I consider that we must definitely accept what Professor Cohn himself, I believe, would approve—namely, that his groups and their genera are simply *assemblages of forms* and may include but a few *real species*, which are represented by phases of growth in each of his form-genera and form-families.

This being the case, it becomes necessary to select generic terms for the designation of the true physiological species. From the widely accepted use of the term *Bacterium*, I am led to adopt it for the generic name of all those Schizomycetes *which under certain conditions of growth present biscuit-shaped plastids capable of active locomotion.*

Accordingly I should place the red organism under consideration in the genus Bacterium as *B. rubescens*, together with *B. lineola* and *B. termo*.

I am not in a position to discuss the whole question, but I think it probable that *Bacillus* is a really distinct genus and that the Spirobacteria are only form-phases of Bacterium and Bacillus.

It appears to me to be an open question whether *B. termo* may not be a diminutive breed or specialised race of *B. lineola*, standing in the same relation to it as does the St. Thomas' maceration-breed of *B. rubescens* (Pl. III, fig. 4) to its larger biscuit-shaped phase (Pl. III, fig. 2). The con-

ditions under which the larger and the smaller form respectively occur in the two cases are similar. The smaller in both cases occurs where nutrition is abundant and the surroundings rendered special by human agency; the larger occurs in natural waters (ponds and streams), where putrescence is going on in the midst of conditions favorable to the maintenance of other forms of life.

The Causes of the Aggregation of Schizomycetous Plastids to form Compound Structures of regular pattern.—I devote a separate paragraph to this subject, not because I have much to say with regard to it, but in order to draw attention to the great peculiarity (already noticed above) in the mode of formation of many of the aggregates of Schizomycetes. There is no doubt that the zoogloea or glœogenous masses of spherical or cavernous (Clathrocystis-like) form increase in bulk by the transverse division of their constituent plastids. But there is reason to doubt whether division along the longer axis of these plastids ever takes place. In the case of the delicate films of Schizomycetous plastids which have been spoken of as the "mycoderma-phase" of growth, it results from observations of the arrangement and connections of the plastids that they must take up their positions to a large extent as the result of a spontaneous movement of apposition —a kind of mutual attraction, similar to that exhibited by mammalian blood-corpuscles when uniting to form "rouleaux."

The operation of this process of adhesion is modified no doubt by the continued growth of the plastids and their self-division at right angles to their long axes.

The definiteness and beauty of the patterns resulting is very striking. Some of the most curious, exhibiting a very elegant combination of curved lines, are due to colourless species of Schizomycetes, and have never, as far as I know, been figured. The most striking of these patterns due to the "fortuitous concourse" of the plastids of *B. rubescens* are those seen in the "regular tesselate" form, Plate III, fig. 5, and Plate XXIII of my former paper, figs. 19 and 21.

Addendum.—I have omitted to describe two additional frond-forms, or aggregates exhibited by *Bacterium rubescens*. The first is not uncommon in very abundant growths of small biscuit-shaped plastids. The plastids are arranged in star-like groups consisting of 10 to 20 plastids, meeting one another at a central point. This form of aggregate I mentioned in my former paper as observed in the case of the acicular form of plastid (see Plates XXII, XXIII, of former memoir,

figs. 2 and 28). It now appears that this *stellate* condition is assumed also by the more common biscuit-shaped plastids.

The second additional form of aggregation was observed very abundantly in Mr. Archer's gathering of *B. rubescens*. It may be described as *cylindrical*. Suppose a number of *stellate* aggregates superimposed and you have a cylinder. These cylinders are often six or seven times as long as they are broad.

On the DEVELOPMENT *of* TEETH. By CHARLES S. TOMES, M.A., Lecturer on Dental Anatomy at the Dental Hospital of London. With Plates IV and V.

FOR many years past it had been thought that, in its broad outlines at least, our knowledge of the development of teeth was accurate; few persons have hence devoted their time to the verification of the current descriptions, and even now, though several years have passed since Kölliker and others first published their results, and proved that inaccuracies of moment existed in the received accounts, the old theories still hold sway in most of our text-books.

This being the case, I propose to give a short outline of the leading facts in tooth development as they are at present known, not confining myself to my own researches (which relate chiefly to the teeth of reptiles, amphibia, and fishes), which are more fully detailed in papers published in the 'Philosophical Transactions.'

In the papers referred to, and in a manual of dental anatomy now in the press, I have given full references to the authors who have of late years contributed to our knowledge of the subject; and I will, therefore, not attempt to do more in the present communication than allude to a very few of their more important papers.

Until within the last few years the researches of Goodsir were taken as the foundation of our knowledge of the development of human and other mammalian teeth, and the generalisations of Professor Owen as authority for the modifications to be met with among reptiles and fish.

The starting-point of Professor Owen's generalisations happened, however, to be just that portion of Goodsir's description which is not quite in accord with the facts since observed, so that subsequent investigations controvert very much that is

laid down in his 'Odontography' and in his more recently published works.

According to the Goodsir theory, the first step was the formation of an open groove (primitive dental groove); from the bottom of this groove free papillæ were supposed to rise up (dental papillæ); while their subsequent enclosure or encapsulation was believed to be brought about by an upgrowth of the walls of the groove, which arched over and enclosed the papilla, the development of an enamel organ being a *secondary* process.

More recent investigations have compelled us to adopt several modifications in this account. In the first place there is never an open groove at any period, and, consequently, there are never any uncovered free papillæ; and, further, there is no process of encapsulation, as there described.

What really takes place is this: from the deep layer of the epithelium (the rete Malpighi) an ingrowth, consisting of a double layer of cells, takes place, burrowing down into the submucous tissue, and looking, in sections transverse to the jaw, like a tubular gland (*c* in figs. 5 and 6). This inflection of epithelium is said to take place uniformly, all round the circumference of the jaw, and thus far there is no indication of the position of the individual teeth (Kölliker, Waldeyer, Thiersch, Frey).

The next stage consists of an active growth of cells which takes place in the deepest end of this inflection of epithelium; while simultaneously, or closely following upon this epithelial development, the tissue immediately subjacent to it becomes elevated at corresponding points, bringing about the condition of things represented in fig. 2; these changes taking place only at those spots where teeth are to be developed. The subjacent tissue forms a conical eminence (dentine papilla, dentine pulp), which is invested above by a bell-like cap of epithelium (enamel organ)—(Kölliker, Waldeyer).

It is to be noted that—

1. There is no breach of surface, but that the whole process takes place in the midst of solid tissue.

2. The enamel organ is in no sort of way a secondary formation, but that its appearance is coincident with, if not antecedent to, that of the dentine papilla.

3. There is not, and cannot be, any process of encapsulation such as was formerly described.

The significance of these results becomes more apparent when the phenomena observed in the jaws of fish and reptiles come to be described; but it may be worth while to point

out how Goodsir may be supposed to have fallen into the error involved in his description.

If a fœtus be kept in spirit the epithelium is very apt to peel off, or to become but loosely adherent. A very slight amount of manipulation would tear open the line of inflected epithelium, thus exposing at its bottom the eminences forming the dentine pulps. These would thus be described as free, uncovered papillæ at the bottom of a deep groove; the error of description is thus very intelligible, and was hardly to be avoided at that date, when the methods of microscopic research were in their infancy.

At this stage we have distinguishable the enamel organ and the dentine pulp, an indication of the future dental sac being visible in the form of prolongations from the base of the dentine pulp, which pass up outside the enamel organ (see *h* in fig. 1). The latter, formed as it was from an ingrowth of the cells of the rete Malpighi, retains for some time its connection with the oral epithelium through a narrow band of cells called the "neck of the enamel organ" (*c* in figs. 1, 2, &c.).

After awhile the neck of the enamel organ becomes broken, and its connection with the oral epithelium lost, while the tooth-sac is completed by the upgrowths from the base of the dentine papilla before alluded to (*h* in fig. 1), arching over the top of the enamel organ and meeting above it.

In the meantime the enamel organ itself has become modified, so that it now consists of a continuous sheet of large cells, extending round its periphery, while its interior has become transformed into a "reticulum" of stellate cells (*f* in fig. 1). The peripheral epithelial cells, where in contact with the dentine papilla, form a regular pavement of columnar or prismatic cells (enamel cells, *e* in the figures), and take the name of "*internal epithelium of the enamel organ;*" while those forming the outer wall of the enamel organ are more rounded, and go by the name of the "*external epithelium of the enamel organ.*"

The enamel is formed by the direct calcification of the cells of the internal epithelium of the enamel organ, the share taken by the other portions of the enamel organ being comparatively insignificant.

Thus far the origin of the tooth-sacs of the temporary teeth only has been alluded to; the permanent tooth-sacs, originating at a much earlier period than has generally been supposed, are developed in a precisely analogous manner, save that the epithelial processes or enamel-germs whence are formed their enamel organs do not spring directly from

the oral epitheliums, but bud out from the necks of the enamel organs of the corresponding temporary tooth-sacs (see fig. 1). Thus the only connection which the permanent tooth-germ as with that of the temporary tooth is through the medium of its enamel germ ; its dentine papilla and sac originate perfectly independently, at a spot which appears to be determined by the position attained to by the enamel germ.

In this manner the enamel organ of each successional tooth-sac is derived from a part of that of its deciduous predecessor; while those teeth which have no such predecessors—*i. e.* the true molars—originate in the following manner:—The enamel organ of the first true molar is derived from the back end of that same epithelial inflection whence sprang all the temporary tooth-germs ; in its origin it, therefore, ranks to some extent with the deciduous teeth, being, like them, derived from a primary ingrowth of epithelium.

The enamel organ for the second permanent molar buds out from the neck of that of the first; that for the third or wisdom tooth from the neck of that of the second (Legros and Magitot).

The development of mammalian teeth had thus been fairly well worked out, and the subject was placed on a satisfactory basis of observation, but little had been done towards the elucidation of the process as exemplified in reptiles and fish.

Professor Huxley, as early as 1853 ('Micros. Journal'), had arrived at some conclusions very far in advance of the knowledge of the time, and uttered a protest against the universality of the papilla theory; Dr. Lionel Beale ('Archives of Dentistry,' 1863) had arrived at a similar conclusion with regard to the teeth of the newt, and, although I cannot wholly agree with his views, had described several points of great importance with entire accuracy. With the exception of these, and a more recent paper by Santi Sirena, the writings of Professor Owen practically held sole possession of the field.

The following may, I think, be taken as a fair summary of Prof. Owen's views (his own words I have quoted in my papers before referred to).

The whole process of development of the teeth in certain fish might be taken to represent an early stage only of the development of mammalian teeth ; thus the teeth of sharks are said to be developed from germs which never become encapsulated: in fact, never pass beyond the papillary stage.

Of Reptiles and Batrachia, on the other hand, he says, that in all cases the papilla becomes enclosed in a capsule; that it never stops short at the papillary stage; and as a deduction from this, that the teeth of all reptiles consist at least of dentine and cementum, enamel being less constant in its occurrence. In his various writings abundant reference is made to the open grooves in which the papillæ destined for the teeth of reptiles originate.

We are thus led to believe that in fish, batrachia, and reptiles there is displayed some sort of progression towards the more complete and more complex process characterising the development of the organs in mammalia; in other words, that the transitional stages of the latter may be taken as representing all that occurs in the former.

But notwithstanding the *à priori* probability of some such relation, I shall have to show that no such generalisations can be drawn—at all events, not at all in the form originally set forth.

At this point I took up the investigation, and by a curious concidence published my results at almost exactly the same time as a German observer, Hertwig,[1] whose observations cover a good deal of the same ground, and whose figures correspond very closely with my own; but although the dates of publication very nearly correspond, my paper, I believe, takes priority.

[1] *Hertwig*, 'Ueber Bau und Entwickelung der Placoidschuppen und der Zähne der Selachier. Jenaische Zeitschrift' (published September, 1874).

Hertwig, "Ueber das Zähnsystem der Amphibien und seine Bedeutung," &c.

'Archiv f. Mikr. Anat. Supplementheft' (published December, 1874).

Chas. S. Tomes, "On the Development of the Teeth of the Newt, Frog," &c., 'Philos. Trans.,' 1875 (paper received July, 1874, read November, 1874.

Chas. S. Tomes, "On the Development of the Teeth of Ophidia" ('Phil. Trans.,' 1875. Received October, 1874, read November, 1874.)

Chas. S. Tomes, "On the Development of the Teeth of Fish" (received March, read April, 1875).

I have only very lately become acquainted with Hertwig's papers; it will be seen that my papers on the teeth of amphibia, &c., were in the hands of the Royal Society, and the abstract published before the publication of his paper; whilst his paper on the development of the teeth of Selachia, was published before my paper, which, however, included also the osseous fishes.

Whilst I am desirous of stating that my results were arrived at entirely independently, and some of them published before his, I must add that he has gone into some questions with much more minuteness than I had; and that although there are a good many points in his paper with which I do not agree, it contains a very large amount of valuable and interesting research.

An observation of my own upon the tooth-germs of the armadillo, and one of Mr. Turner's upon that of a fœtal narwal, had served to indicate the probability that within the limits of the mammalia class the presence of an enamel organ would be found universal, as in neither of these animals is there any enamel upon the completed tooth.

My own further researches upon the tooth development of fish, batrachia, and reptiles has shown that the presence of an enamel organ is quite universal, and that the ingrowth of epithelial cells (enamel germ of Kölliker) is the earliest recognisable step towards the formation of any and every tooth-germ.

A common English lizard may be taken as our example from the class of reptiles. I have never had an opportunity of observing the development of the first-formed tooth-germs, but the manner in which the successional teeth are formed may be very completely worked out.

In man the tooth-germs (even of the permanent teeth) are formed during the fœtal period, and they are at first very close to the epithelial surface. But in reptiles, where the development of new teeth is going on throughout the creature's life, the new germs are formed at a considerable distance from the surface. Hence the inflection of epithelium penetrates to a far greater depth before it reaches the spot where the dentine papilla is to be formed (*see e* in fig. 3), so that it plays an even more conspicuous part than in a mammalian jaw. When it has reached through the whole thickness of the soft parts, nearly to the bone, a set of changes closely similar to those previously described takes place; fig. 2 represents a young tooth-germ of a lizard. In its further development some points of difference which would serve to distinguish it from mammalian tooth-sacs arise. In the first place a less definite capsule is formed; the surrounding connective tissue becomes pushed on one side and so forms a slight adventitious capsule, but this is all.

The enamel organ also in its further development becomes differentiated into an internal epithelium (enamel cells) and an external epithelium, but these are at no period separated from one another by any loose network of stellate cells (the reticulum). The dentine papilla presents no marked divergence from the characters met with in those of mammalia.

The tooth when completed moves up into position, tooth-sac and all; but it becomes fixed to the bone by a rapid development of coarse bone which takes place outside the limits of the sac, and not by a calcification of a part of its own capsule, as has been generally supposed.

In the order Ophidia an interesting and eminently characteristic arrangement is met with. A very large number of successional tooth-germs are always in course of preparation; thus there are usually about eight or nine successional poison fangs in various stages, and six or seven sacs at the base of the ordinary teeth (see e_1, e_2, e_3 in fig. 4).

These successional sacs are arranged together in a sort of nest, within a connective-tissue capsule, an arrangement likely to prove advantageous when the mouth is dilated to swallow prey; each one consist of an enamel organ, a dentine pulp, and a feeble connective-tissue investment.

The youngest tooth-germ is to be found at the bottom of the series (e_4 in fig. 4); it originates from a budding down of an 'enamel germ" from the neck of the enamel organ of the tooth-germ next above it, and the subsequent or coincident formation of a dentine papilla (see *i* in figure); not only do the tooth-germs of all Ophidia possess enamel organs, but these actually form a thin coating of enamel; and Professor Owen was mistaken in supposing that enamel was absent, but that the teeth of "all Ophidia consist of dentine and cement." No Ophidian tooth, so far as the examination of a very large number of specimens can decide, possesses cementum at all.

In Batrachia the process is in all essentials similar, though it is subject to modification according to position. In the newt the chain of successional tooth-sacs is very beautifully seen (see fig. 6), an enamel-germ, which as yet has no corresponding dentine-pulp, extending beyond the youngest of the series (*c* in fig. 6).

The tooth-sacs of the newt have the peculiarity, first noted by Dr. Lionel Beale, that they have no capsular investment whatever. They consist solely of aggregations of cells, and under pressure readily break up; the enamel organs form a spear-like point of enamel upon the teeth, but the enamel does not extend down upon the sides of the teeth.

In the frog the edentulous lower jaw closes within the upper teeth, and so little space is left that an arrangement like that seen in the newt becomes impossible. In fig. 5 an enamel germ is seen at the inner side of the base of the tooth in place; as the new tooth-sac attains to any considerable size, space is made for it by the absorption of the base of the older tooth, into the pulp cavity of which it finally passes, recalling in some measure the manner of succession met with in the crocodiles. It is also a question whether the new enamel germs really do spring from the necks of the enamel organs of older tooth-sacs, or whether they originate *de novo*

from the oral epithelium; though analogy would lead one to infer the former to be the case, the small amount of available space and the speedy migration of the growing sac render it difficult to positively decide this point.

The sharks, in which Professor Owen was of opinion that tooth-development stopped short, as it were, at the papillary stage, present an instructive parallel with the condition of things seen in either the newt or the axolotl.

The series of dentine papillæ, save that they originate from a continuation of the same sheet of mucous membrane, have no genetic conection with one another; on the other hand, the enamel organs of the teeth of successive ages form a chain the continuity of which is never broken by that subsequent individualisation of the several tooth-germs which takes place in reptiles and mammals.

The teeth of sharks are seen, in a fœtus just on the point of being hatched, to be merely highly-developed dermal spines, with which they are directly continuous, as there is no definite lip at this period, and the spinous skin is seen turning in over the jaws. Hertwig has worked out, more carefully than I, the development of these dermal spines, which he declares to be in all respects similar to that of the teeth; he considers the excitation of greater use to be adequate to account for the greater development of the dermal appendages where the skin passes over the jaws.

In the development of the shark's tooth we find, just as in every other creature, that there is a production of an enamel germ extending beyond the youngest one already existing (*e* in fig. 7); that opposite to this the submucous tissue becomes elevated into a dentine papilla. But there is nothing like a production of free papillæ, which become subsequently and secondarily enclosed or encapsulated; and the production of the enamel organ is in no sense secondary to that of the dentine papilla. When the parts are undisturbed the flap of mucous membrane (*k* in fig. 7) which covers in the teeth is in slight connection with the jaw and teeth; it may be very readily torn away, but it is only so displaced by violence, and it is not, as was generally supposed, a free flap, which merely fitted against the developing teeth.

In some of the elasmobranchs a distinct coat of enamel is deposited; in others it amounts to a mere glaze, but from the appearance of the enamel cells, which in other cases proves to be a tolerably reliable guide, I should infer that they do discharge a function, and that the outer structureless film on the tooth, of Lamna for example, is to be regarded as enamel.

If one were desirous of representing diagrammatically the development of the teeth of a shark and a newt, one would draw almost the same thing, save only that in the former the several tooth-sacs are somewhat less individualised.

Hertwig entertains peculiar views as to the homologies of the parts of placoid scales and sharks' teeth, regarding their basal portions as *cementum;* his paper has been in my hands so short a time that I am not prepared to express an opinion upon this matter, but if we admit Hertwig's views as to the basal part of a selachian tooth, we must probably extend the term cementum to a large number of structures never hitherto ranked as dental tissues at all.

Passing from the sharks and rays to the osseous fish, I believe that a broad distinction may be laid down. In all mammals, reptiles, batrachia (the frog?), and elasmobranch fishes, successional teeth are derived, though the medium of their enamel organs, from their predecessors. In all osseous fish which I have examined, successional teeth appear to be produced *de novo*, *i.e.* from new inflections of the oral epithelium (see fig. 8, representing a young tooth-sac of a mackerel).

With this exception the process is the same; there is the same formation of the enamel germ from the oral epithelium, which is transformed into an enamel organ over the uprising dental pulp; the latter becoming always calcified, the former sometimes dwindling away without any enamel formation taking place.

There are many fish (*e.g.* eels) which have spear-like terminal points of enamel, but no enamel on the sides of the teeth; in the tooth-germ the enamel organ is, nevertheless, coextensive with the dentine pulp, though its internal epithelium or enamel cells are only largely developed over the point where enamel is to be formed, and are small and dwarfed over the rest of the tooth (see fig. 10).

From what has already been said it will be seen that there is considerable uniformity in the manner in which tooth-germs are developed, and that it is quite impossible to maintain the generalisations that the teeth of fish and reptiles typify any certain transitory stages in the development of mammalian teeth, &c. And it will also be seen that Goodsir's original error of observation, small though it was, becomes vastly magnified when his views are stretched to make them apply, for instance, to the common snake (see fig. 4).

Although, morphologically, as Professor Huxley reminds me, an inflection of epithelium is not a very different thing

from a groove lined by epithelium, yet any description which spoke of the successional teeth of a snake, or yet more of its poison fangs, as being developed from free papillæ in a groove, would be somewhat wide of the mark.

My observations have led me towards certain other questions of interest. In the first place, are we still to regard dentine as the most, cementum as the next, and enamel as the least constant of dental tissues? According to the older accounts of development such must almost necessarily have been the case; but is it really so? The Ophidia, at all events, amongst whom cementum has been supposed to be of universal occurrence, have none, but have enamel instead (see 'Phil. Trans.,' 1875), and I am inclined to think enamel at least as constant, if not more so, than cementum.

The true homologies of the whole enamel organ were first pointed out by Professor Huxley in the valuable paper referred to; with regard to the several parts of the enamel organ, the reticulum appears to be peculiar to the mammalia; at all events it is certainly not an essential part, as enamel is formed in countless teeth in which it was never present. Indeed, some recent observations of my own upon the development of the poison fangs of snakes would seem to point to the inference that it is essentially a retrograde metamorphosis of a part of the enamel organ. It has already been mentioned that the concidence between the position of the large enamel cells in an enamel organ such as that of the eel (see fig. 10) with that of the enamel cap afterwards formed would, so far as it goes, support the conversion theory of the development of enamel; the more so as it is generally possible to judge from the extent to which this internal epithelium of the enamel organ is developed, whether enamel will or will not be deposited upon any tooth; Hertwig, however, believes that enamel is not formed by direct calcification of the enamel cells, but by a secretion from them; he also reasserts the existence of the membrana preformativa,[1] but not, in my opinion, upon satisfactory grounds.

The development of cementum is a subject by no means satisfactorily worked out; how far it has relation to the tooth-sac capsule, and how far to the periosteum and connective tissue outside the sac, I hope to be able to determine; but it appears to me that its presence is almost always asso-

[1] After a careful review of the literature of the subject, and not a few attempts to demonstrate it, I cannot think that the presence of a basement membrane (*M. preformativa*) has been established, and the appearances described are, to my thinking, capable of other interpretations more in accord with what I have myself seen. Waldeyer also denies its existence.

ciated with implantation in a socket, though it may be in a very incomplete one.

To sum up the results of the foregoing observations:

1. All tooth-germs whatever consist of at least two parts—an enamel organ and a dentine pulp; a capsule may or may not be present.

2. The first step recognisable is invariably the ingrowth of epithelium, *i.e.* an enamel germ.

3. Enamel germs of successional teeth in mammals, reptiles, batrachia, and elasmobranch fish are derived from the necks of the enamel organs of their predecessors; the respective dentine pulps originating independently, the enamel germs constitute the sole connection between dental germs of various ages.

4. In all the osseous fish examined by me the several enamel germs originate *de novo* from the oral epithelium; this may, perhaps, be the case in some other creatures, but I have never seen it.

5. The enamel organ, invariably present, consists primarily of external and internal layers of epithelium; the former atrophies, the latter grows largely if the tooth is to be capped with enamel; otherwise it also atrophies, but at a later period.

6. The stellate tissue of the mammalian enamel organ is obviously non-essential; indeed, it is probably a retrograde metamorphosis.

7. Dentine pulps originate independently of each other, at points in the submucous connective tissue apparently determined by the position attained by the ingrowing enamel germs; in structure they are singularly uniform in all animals.

8. There is no example of a dentine pulp originating as a free papilla upon the surface; on the other hand, as in the snake, the process often takes place at a great depth below the surface.

9. The terms "papillary," "follicular," and "eruptive stage" should be quite abandoned, as tending to perpetuate a somewhat mistaken hypothesis.

10. Enamel is obviously a metamorphosed epithelium;[1] dentine belongs to the submucous connective tissue; the homologies of cementum have yet to be determined; but the basement membrane known as membrana preformativa has probably no significance in the process, if indeed it has any existence.

[1] This is particularly obvious in Fish and Amphibia, and Heriwig has given figures of developing placoid scales in which the rete Malpighi, but little specialised into an enamel organ, appears to become calcified into enamel.

An Account *of* Professor Haeckel's recent Additions *to the* Gastræa-theory.

By Professor E. Ray Lankester, M.A., F.R.S. With Plates VII, VIII, IX, X.

In the last numbers of the 'Jenaische Zeitschrift' Professor Haeckel has continued his important discussion[1] of the facts of development in connection with the significance of the primitive germ-layers as the key to animal genealogy. Some useful terms are introduced by him to designate phenomena which have been more or less clearly recognised during the development of this subject in the past three years, and further, some original observations on the development of particular animals are given. By the courtesy of Professor Haeckel I am able to place before the readers of this Journal four of the instructive plates which illustrate his recent contributions to the Gastræa-theory; and I propose to give some account of his views, without, however, committing myself to complete agreement with them.

Palingeny and Cenogeny.—These terms were introduced by Haeckel in his Anthropogeny, and will no doubt be found useful. In the ontogeny, or individual development of living forms, we have been accustomed under the new régime of Darwinian embryology to distinguish those forms and structural dispositions occurring in the course of development from the egg, which appear to be due to heredity, from those which appear to be due to adaptation.

It has been recognised[2] that there are two distinct tendencies to be estimated in the process of ontogeny: the tendency to *recapitulation* of the complete series of ancestral forms, the effects of which Haeckel terms Palingenesis, and the tendency to adaptation to present conditions resulting in the suppression and hurrying over of steps in the recapitulation and the development of special larval or embryonic organs. The effects of this tendency are what Haeckel sums up as Cenogenesis. Cenogenetic phenomena have hitherto been spoken of as 'falsifications' of the recapitulation, as 'abbreviations' and larval 'adaptations.' In the series of ontogenetic phenomena presented by an organism, it is clear that it is the palingenetic portion of them which have most value for the *morphologist*, whilst to the *physiologist* the cenogenetic are especially interesting.

Heterochrony and Heterotopy.—Ontogeny being essentially

[1] See this Journal, 1874, for a translation of Haeckel's essay, "The Gastræa-theory."

[2] See "Primitive Cell-layers of the Embryo," 'Annals and Mag. Nat. History,' May, 1873, p. 322.

a series of phenomena of space-relations succeeding one another in definite order, it admits of variation (and consequently of appropriate discussion) in relation to the two elements of space and time. The perturbations in the palingenetic evolution of an organism which may be classed as cenogenetic, are necessarily either dislocations of the palingenetic phenomena, in so far as regards *time* or in so far as regards *space*. Hence we may with Haeckel very conveniently speak of such phenomena as *heterochronous* or *heterotopous* as the case may be.

The appearance of an organ in an embryo at an earlier period (that is to say, at a period of lower development of its other organs) than that at which we have reason to believe this organ made its appearance in the Phylogenesis or ancestral development of the form in question, is a frequent example of heterochrony. As examples, Haeckel cites the early appearance of the notochord, of brain and eyes, of the gill-slits, and of the heart (before the vessels) in Vertebrata; the early development of the limbs, and of the segments (of the primitive stripe) in Arthropods generally; of the tracheæ of the Tracheata, and the liver of the Crustacea. The Mollusca furnish instances in their early developing otocysts, and, I may add, in the early appearance of the 'shell-gland' or invagination of the mantle, the Gasteropods in their lingual rasp, and the Echinoderms in their calcareous skeleton.

Similarly we have examples of heterochrony which consist not in the early but in the *late* appearance of inherited ancestral traits of organisation. This kind of dislocation is very generally seen in the late development of the sexual glands in all Metazoa, and in the postponement of the completion of the alimentary canal, even though its rough materials take up their position at the normal palingenetic period. A striking example is, according to Haeckel, the late formation of the septum of the auricles in the embryonic heart of the higher Vertebrata, which is subsequent to that of the septum of the ventricles. In the phylogeny of the Vertebrata, on the contrary, it is clear that the two septa originated in the reverse order, as the Dipneusta, the Amphibia, and the Reptiles demonstrate.

Heterotopy is even a more important element of the cenogenetic modification of the primary palingenetic phenomena. I have myself insisted on the fundamental importance of this common process in the comparison of the delaminate and invaginate forms of Gastrula or Planula, and in the comparison again of the epibolic and embolic forms of invagination (see 'Ann. Nat. Hist.,' May, 1873, p. 328,

and 'Proceedings of the Royal Soc.,' 1874), and further, in relation to the development of organs in the middle layer of the germ which are palingenetically hypoblastic or epiblastic, as, for instance, the notochord of Vertebrates (shown by Mr. Balfour to develop from the hypoblast in the sharks, probably its true palingenetic position), and the nerve-ganglia of Loligo (shown not to develop from epiblast as nerve-centres should do in undisturbed palingenesis; see 'Quart. Journ. Micr. Sci.,' 1875, p. 46).

As Haeckel observes, first and foremost in this connection are the 'wandering' of cells and shifting of groups of cells in the earliest stages of development—small shiftings taking place in an early stage—such, for example, as the interpenetration in limited points of the two primitive cell layers, the ectoderm and endoderm, or epiblast and hypoblast which constitute the critical developmental form of all higher animals, the Gastrula or Planula. To this heterotopic movement of cells in an early phase of development, possibly occurring even in the first stages of cell-formation or cleavage of the egg, it is very possible that large dislocations in the seat of development of organs, manifesting themselves at a later period, should be ascribed. Haeckel would thus account for the assumed mesoblastic origin of the sexual glands in certain of the higher animals, holding, as do most embryologists, that these glands are palingenetically not part of the *middle* layer of the germ, but part either of the epiblast *or* of the hypoblast.

Considerations of the same kind enable us to explain in a general way the diversity of origin which the middle layer presents in different groups of animals. At the same time it must be admitted that accurate information as to even the apparent origin of the mesoblast in a large number of cases is not to hand, and the statements of authors worthy of credence vary in reference to one and the same animal. Haeckel, as is well known, holds that the mesoblast is phylogenetically formed of two layers, respectively the deep layer of the ectoderm and the deep (*i. e.* sub-epithelial) layer of the endoderm. Its apparent development entirely from hypoblast or endoderm in the Vertebrates he regards as due to the heterotopy of the ectodermal portion,[1] and the splitting which occurs

[1] A possible explanation of such a case as the disappearance of the ectodermal factor of the mesoblast is suggested (I venture to submit) by a comparison with the phenomena of atrophy, such as we observe in the cases of rudimentary organs and the final suppression of such organs. Atrophy very generally is accompanied by a *transference* of the nutrition proper to the atrophied part to a neighbouring or substituted structure, and when the transference reaches its full development and extends back into the period of early embryonic life, even the primitive cells which formed the first outlines of an organ or of a germ-layer may be totally

in the Vertebrate's mesoblast giving rise to the cœlom of that group (pleuroperitoneal cavity) he regards not as the primitive separation of the two deep layers (of ectoderm and endoderm respectively) by which he supposes the cœlom to have originated phylogenetically, but as a secondary splitting following upon their heterotopous fusion. In this connection it is important to observe that we have not *much* evidence in favour of the view that the cœlom *did* phylogenetically arise by the splitting of the two factors of the mesoblast, that is to say, was a schizocœl (Huxley). *Possibly* it was in origin an outgrowth of the alimentary canal, a gastro-vascular apparatus or enterocœl (Huxley), as its development in Echinoderms, Terebratula, and Sagitta tends to prove.

As the important general result of the two classes of cenogenetic phenomena, which Haeckel distinguishes, viz., Heterochrony and Heterotopy he points out that in the progress of time those organs are more and more strikingly brought into the foreground of an ontogeny which are especially characteristic and important for the stem, class, or order to which

suppressed. From one point of view, which is not here touched on by Haeckel, the most important developmental phenomena are those of Hypertrophy and Atrophy. In organic evolution (phylogeny) organs do not arise *de novo*, but are produced by the moulding influence of gradually increasing hypertrophy of this part and atrophy of that. In ontogeny such hypertrophies or atrophies may present themselves in the course of the developmental recapitulation in their due order, the order in which they appeared in the series of ancestors. They then fall under the head of 'palingenetic' phenomena. But if they occur earlier or later than is their proper place they fall under the head of heterochronous cenogenesis. It is clear then that cenogenesis in divisible into heterotopy, heterochronous hypertrophy (*positive* growth) and heterochronous atrophy (negative growth). Thus the atrophy of organs which have made their appearance in the embryo but disappear in the adult is very generally part of the palingenesis. On the other hand, the total or very early suppression of organs in the embryo such as the notochord absent from most Tunicate ontogenies, the gill tufts absent from the ontogenies of Amnionate Vertebrata, which should appear if the palingenesis were carried out though to disappear before the adult form is attained, are examples of the heterochronous atrophy of cenogenesis.

It is to be noted with reference to the terms palingenesis and cenogenesis, that they can only be used in relation to specified tracts of the organic pedigree. The cenogenetic phenomena of an early ancestor of this or that organism necessarily *become part of the palingenesis* of its descendants. For instance, the development of the mesoblast from the hypoblast exclusively may be as Haeckel supposes, a cenogenetic phenomenon in vertebrata relatively to the ancestors of an earlier grade of development. Once, however, acquired by the earliest Vertebrates, it becomes in other Vertebrates relatively to them a palingenetic phenomenon. Thus in birds as representatives of the Vertebrata, we may speak of it as cenogenetic, but in birds as compared with Vertebrates of the grade of development of the sharks, it can only be spoken of as palingenetic. With regard to atrophy see my paper on 'Development of the Cephalopoda,' this Journal, 1875, p. 46.

the developing organism belongs. Thus in the Vertebrata the notochord and the branchial slits are out of all proportion early in their appearance, and large in relation to their adult size and the size of other organs; whilst, on the other hand, those organs are more and more pushed into the background which have the most general significance for all Metazoa. Hence above all other organs we find the primitive alimentary canal,[1] or archenteron, and the primitive mouth[2] suffering in this respect in so far as their primitive form is concerned. Hence too the simple, primordial Gastrula, which arises by the invagination of a perfectly simple sac, the wall of which consists of a single layer of cells (Blastula), is especially preserved in the most faithful way by the lowest, most indifferent, and oldest forms of the different groups (for example, in the ontogeny of Gastrophysema, Monoxenia, Sagitta, Phoronis, Argiope, Terebratula, Uraster, Toxopneustes, Ascidia, Amphioxus).[3]

Palingenetic plastic yelk and cenogenetic food-material.—The great importance of distinguishing these two elements and their share in early developmental phenomena is insisted upon by Haeckel. He holds that (as I have maintained in relation to the developmental phenomena of the Mollusca) the food-material thrown in to the primitive egg-cell disturbs and clouds the subsequent development in the most profound manner, hindering and concealing the full unfolding of the palingenesis. In all cases Haeckel maintains that the egg is palingenetically a single cell and that the food-material is a cenogenetic addition or adaptation.[4]

[1] Since we have no words equivalent to the German Darm and Urdarm, we may use the Greek equivalents enteron and archenteron.

[2] Or 'blastopore.'

[3] The examples cited by Haeckel do not appear to me altogether to warrant this conclusion. Sagitta, Terebratula, and Toxopneustes are not generally admitted to be either indifferent or archaic types of the groupsto which they belong. And even if they were it is not easy to follow out the reasoning by which it is concluded that they should therefore be expected to exhibit the simplest form of gastrula, for although assumed to be low in their respective groups, yet there is a very long distance between any of them (except the Sponges) and the ancestral gastrula, a distance sufficient to allow of the development of countless heterotopisms and heterochronisms, and without doubt sufficient to allow of the development of that particular cenogenetic phenomenon which beyond all others is efficient in obscuring the gastrula form, viz. the addition to the egg-cell of food-yelk, deutoplasm, or 'food-material.' I pointed out the important influence of this accessory yelk in my original paper on this subject, 'Ann. Nat. Hist.,' May, 1873, p. 328, and in subsequent embryological writings.

[4] From one point of view the admixture of food-material with the protoplasmic egg-cell may be regarded as a palingenetic survival of the 'amœboid form of nutrition,' in which solid food is taken bodily into the living sub-

The four chief types of Egg-cleavage and of Gastrula-formation.—According to Haeckel we may distinguish in the processes of egg-cleavage and the immediately subsequent phenomena of development which result in the assumption of the essential gastrula-form—four types, one primitive (the true palingenetic series of changes), whilst the other three are cenogenetic modifications of the process brought about by variations in the quantity and mode of admixture of the food-material. In each type five stages of development may be distinguished, leading as far as the completion of the Gastrula form—namely, the Monerula or fertilised egg after the loss of its ovarian nucleus or germinal vesicle; 2, the Cytula or egg with newly formed cell-nucleus; 3, the Morula or mulberry form (Polyplast), a spherical agglomeration of simple equi-formal cleavage-cells; 4, the Blastula or blastosphere, a hollow one-cell-layered vesicle, formed by the accumulation of fluid within the Morula; 5, the Gastrula, a simple two-cell-layered sac, whose cavity, bounded by the two primary germ-layers, opens to the exterior by the Archistom (or blastopore).

To the five stages belonging to the simplest type (that which he considers strictly palingenetic) Haeckel gives the prefix 'archi.' We thus have a series designated as follows:—Archimonerula, Archicytula, Archimorula, Archiblastula, Archigastrula. To the second type the prefix 'amphi' is assigned, and we thus have a corresponding series leading to the Amphigastrula. The third type have 'disco' as their distinctive name-component, whilst 'peri' marks the fourth group. We shall take these into consideration one by one, merely remarking to begin with that the Archiblastic and Amphiblastic series correspond to two modifications of what have long been called Holoblastic eggs; whilst the Discoblastic and Periblastic series are two extreme forms of Meroblastic eggs. Haeckel is careful to point out that though these four types can be conveniently erected as a means for arranging our conceptions, yet that they really are not sharply cut off from one another, but as we should expect there really are transitions in blastoderm-formation

stance of the sarcode mass. It is, however, no doubt most conducive to a clear appreciation of the facts of the case to regard it as belonging to a group of structural phenomena which are to be distinguished from the ultimately resulting embryonic phenomena classed as heterotopy, heterochronous hypertrophy and heterochronous atrophy, and may be designated 'matrificial' (like artificial). These matrifacts are often clearly the antecedents of embryonic cenogenesis. Such are besides the added food-material, the egg-envelopes. Structural phenomena proper to the protoplasm of the egg-cell itself may be called 'ovificial' or 'ovifacts.'

connecting the Archiblastic with the Amphiblastic and with the Periblastic, and the Amphiblastic with the Discoblastic and Periblastic. To the student of Embryology it will be clear enough in a general way that these terms are meant to imply, 1st, ARCHIBLASTIC, the primitive egg-segmentation into *equi-formal* cleavage spheres, and the subsequent assumption of the simple two-cell-layered gastula-form with or without apical orifice, either by invagination or delamination; 2nd. AMPHIBLASTIC, the egg-segmentation into inequi-formal cleavage-spheres, one portion of which is charged with the matrificial food-material, and becomes overgrown by the smaller cells free from food-material (epibolé, Selenka). 3rd. DISCOBLASTIC, the segregation of a small disc or klastic patch from the rest of the egg, in which disc alone the cleavage process is carried on. 4th. PERIBLASTIC, the segregation not of a disc, but of a complete superficial layer of klastic material around the inert food-material.

THE ARCHIBLASTIC TYPE.—Examples of this are met with among the Cœlenterata in Sponges, Hydroids, Medusæ, and Corals; among Worms, as in the case of Sagitta and Phoronis; among Molluscs, as in the Brachiopoda and some Lamellibranchs and Gastropods; among Echinoderms generally; among certain low forms of Arthropoda, as for instance some Branchiopoda and perhaps the Pteromalina (Tracheata); among Vertebrates, in the case of Amphioxus and some Ascidians.[1] Haeckel gives the development of a very simple form of polyp, as typical of the Archiblastic type. This very curious little polyp, which he names Gastrophysema, is similar to the Haliphysema of Bowerbank, and appears even in its adult condition to get no further than the Gastrula stage: it is a true Gastræa. It was originally described by Carter as Squamulina scopula, that observer having taken it for a Polythalamian.

In the plates which we reproduce no figure is given of the Archimonerula, Archicytula, Archimorula, which the reader can easily picture to himself; but in fig. 20 we have the Archiblastula of an Actinia, in fig. 21 its Archigastrula formed by invagination; in fig. 29 the Archiblastula of Limnæus is given, and in figs. 30 and 31 its Archigastrula. Fig. 17 gives the Archigastrula of a Calcareous Sponge (Asculmis), fig. 22 that of a Medusa (Pelagia), fig. 23 that of a worm, Sagitta, fig. 25 that of a Brachiopod (Argiope), fig. 33 that of an Echinoderm (Asteracanthion).

Haeckel holds (and this is an important point for theoretical

[1] Professor Haeckel is not responsible for the classification here adopted of the Tunicata under the great group of Vertebrata.

consideration) that the Archigastrula is *always* what I have termed an 'invaginate Gastrula or Planula,' and, of course, since the cells are of equal size, and the invagination is a real pushing-in, it corresponds with my 'embolic invaginate' type; the 'epibolic invaginate' type, where the smaller cells of the blastosphere grow over the larger cells being classed under the Amphiblastic type. Haeckel is inclined to doubt whether such a thing as a 'Delaminate Gastrula or Planula' ever is formed; that is to say, he does not think that the observations are satisfactory in which the endodermal layer of cells have been stated to arise by cell-division on the inner surface of the blastosphere.[1] Very probably careful observations would show in such cases an invagination at a *very early period*. We are in want of observations (by means of section-cutting) on the development of the Hydroid polyps. But even if delamination does occur it can only be regarded as a heterochrony. The fate of the 'Urmund,' as Haeckel calls it, the orifice of invagination or 'blastopore,' as I prefer to say, is not discussed by him. It is one of the points upon which the whole superstructure of the Gastræa theory rests, as I have pointed out (this Journal, April, 1875). Haeckel, however, insists on the importance of recognising the rim or margin of the blastopore, which he calls *Properistoma*. It is, he holds, identical with the much discussed and highly important 'Randwulst' or 'Keimwulst' of the discoblastic type of development (Chick, Shark, Cuttle-fish). It is here, in the annular space between Entoderm and Exoderm, that the first cells are separated from the two primary germ-layers to give rise to the mesoderm, or mesoblast.

The Amphiblastic type (Pl. X).—The most familiar instance of this type of development, which consists essentially in the unequal size of the cleavage-cells, a portion of which are large and charged with food-material, is seen in the frog and other Amphibia, also in Petromyzon and Accipenser. Probably the Dipneusta and placental Mammals belong to this category. The greater number of the Mollusca are amphiblastic, probably even some Cephalopods (the commoner forms of which are discoblastic) and some Brachiopods (others being archiblastic). Among the Arthropoda amphiblastic development appears to occur only in some of the lower Crustacea and Tracheata. Amongst the Echinodermata only certain Asterida and Holothurida, with a so-called 'direct' or 'abbreviated' development, present the amphiblastic type. A large majority of the Worms are to be reckoned here, and amongst

[1] It does not seem possible to explain Fol's observations on the development of Geryonidæ as indicating anything but a true 'delamination.'

the Cœlenterata, or Zoophytes, there are some excellent examples of the type; for instance, among the Ctenophora and Siphonophora, as well as in Corals and Sponges.

In Plate X two examples of the amphiblastic mode of development as far as the Gastrula stage are given; the upper series relates to the Annelid *Fabricia*, one of the Sabellidæ, and the lower to a Gasteropod, probably a *Trochus*. They were both studied by Haeckel at Ajaccio. He recommends the use of carmine and hæmatoxylin as staining agents, the embryo being rendered transparent by glycerine. The reader is referred to the explanation of the plates for further details.

The amphiblastic type of development presents a great range of variation in respect of the amount of food-material incorporated with that portion of the Monerula which is to give rise to the endoderm-cells, and an accompanying variation in the number of cells produced by its cleavage and the rate at which it cleaves. In this way heterochronies are brought about, the unencumbered ectoderm proceeding more rapidly on its palingenetic development than does the endoderm.

All amphiblastic Gastrulæ are not epibolic, that is to say, brought about by the growth or circumcrescence by the ectodermal cells over the larger entodermal cells. In some (for instance, Unio, Figs. 26, 27, 28) the relative growth is such as to have the same appearance of embolé (or entobolé), which is observed in the Archiblastic series. As I pointed out (loc. cit.) there is no sharp line to be drawn between embolic and epibolic developments, but they are connected by transition forms. Haeckel endorses this view, and further, draws attention to the variation, which I have also insisted on, in the share which the primitive endoderm cells take in the production of the permanent alimentary canal. It is impossible to say, with the observations at present before us, to what extent these variations are exhibited in the various groups of animals. In some cases endoderm-cells are absorbed in the process of development, never taking part in the formation of the wall of the primitive enteron (archenteron). Such cells may be inclosed *within* the enteron or lie outside its walls. Further, it appears (*e. g.* in Limnæus and Pisidium) that the archenteron is sometimes not directly converted into the alimentary canal of the adult, but that large portions of it are absorbed, or, as in Echinoderms, Sagitta, Terebratula, &c., give rise to a body-cavity. Haeckel distinguishes the permanent enteron as metagaster. We may call it the 'metenteron.'

The problems here pressing for solution are of the greatest consequence. The whole question of the nature of the cœlom

or body-cavity is involved, and on this as on the equally important question of the persistence of the blastopore Haeckel maintains a discreet though disappointing silence.

The Discoblastic type.—The essential feature in this type is in the overloading of the egg with food-material. It is led up to by many cases among Mollusca, which present the amphiblastic type so far, that the portion of the egg-cell which contains the unassimilated food-material cleaves once, or possibly twice, but ceases after that to take part in the process of cell-formation and serves only as a reservoir of nutriment. Rarely (as I have suggested in the case of Aplysia, in a paper received January, 1874, and published in 1875 in the 'Phil. Trans.' by the Royal Society, and as Fol has since recorded in the case of Pteropods) the large segments charged with food-yelk segregate at a later period of development a certain number of cells which give rise to the hypoblast, or as appears from Fol's observations, sometimes to part of the epiblast. This late segregation may be compared to the Periblastic formation of the blastoderm to be described below. Haeckel points out that in the Discoblastic type we have cases (especially among osseous Fish, one of which, a marine Gadoid in all probability, he describes and figures in detail) in which the discoidal cap of egg protoplasm or formative-yelk separates *entirely* from the food-material, which remains perfectly homogeneous, and never gives rise to any cells, after the separation of the cleavage-patch or disc. The growth of this over the relatively huge mass of food-material he describes minutely, and considers that an ingrowth of the first cap of cells takes place all round its periphery—the well-known 'Randwulst' of the osseous fish's and chick's development. The result is the formation of a Discogastrula perched on a mass of food-material, which mass blocks or fills up as it were the blastopore or 'Urmund.' The Discogastrula separated from its mass of food-material is seen in fig. 50. Intermediate stages are admitted by Haeckel in which the food-material is not pure, but still contains formative material, which it separates during the growth of the Discogastrula. The cells thus formed are, he says, partly converted into connective tissue, partly into blood-cells. He regards them as belonging to the endoderm. Such are the cells described by Goette in 1874 in the development of the chick, by Balfour in 1874 in the Shark, and by myself in 1873 in Cephalopods ('Ann. Nat. Hist.,' February, 1873, p. 82). To these I subsequently applied the name of 'autoplasts' as opposed to the 'klastoplasts,' which are the products of the segmentation of the cleavage-disc or cap.

Haeckel is very careful to insist on the unicellular character of the Discomonerula and Discocytula, even where it attains the large size seen in Birds. In his support of Gegenbaur's view advanced so long ago as 1861, he will be joined by most embryologists of to-day. He takes occasion to express his objections to the 'parablastic theory' of His, which I may say does not appear to me to be really supported by the fact which His recently quotes from my observations on the ovarian egg of Loligo and Sepia ('Phil. Trans.,' loc. cit.). The cells which are to be found in the ovarian egg of these Cephalopods, and which have passed into it from the cell-lining of the egg-capsule (granulosa), are not in such a state as to lead one to suppose that they are capable of development. They are, as Haeckel insists, passive nutritional material.

The invagination of the disc-like morula in the discoblastic type, to give rise to the Randwulst or Properistoma (blastopore-margin) is a fact of great importance, which Haeckel attempts to illustrate in other vertebrate forms besides the osseous fish, in which he observed it with care. The diagrams (figs. 41 to 54) serve to explain these views, which the reader may compare with those advanced by Mr. Balfour in this Journal, July, 1875.

The origin of the mesoblast in his marine osseous fish Haeckel did not satisfactorily determine. It appears, however—a fact on the general importance of which, as above mentioned, Haeckel insists—that it first makes its appearance in the Randwulst (blastopore-margin), and is, he believes, traceable to delamination from the ectoderm (epiblast), and *also* to delamination from the endoderm (hypoblast). The first portion (Hautfaserblatt[1]) has a *bilateral* symmetry, gives rise to the so-called 'protovertebræ,' and corresponds to the bilaterally symmetrical commencements of mesoblast, which Carl Rabl has observed in Limnæus, and which are characteristic, according to him, of all the Bilateria (that is to say, the Vermes and the four great types connected with them). The second portion, which comes from Hypoblast, consists of a layer of hypoblast cells and of amœboid cells of very active movement, which wander between spaces in the hypoblast and spread themselves partly on the surface of the yelk and partly in the embryo itself. They give rise to blood cells, connective-tissue cells, and pigment cells. The outer layer of hypoblast cells and these amœboid cells Haeckel considers as the Darmfaserblatt.[2] He lays especial emphasis on the fact that this set of cells do *not* have a bilateral arrangement pri-

[1] Hypoderon or hypoderic cell-layer.
[2] Hypenteron or hypenteric cell-layer.

mitively, but arise equally and simultaneously over the whole periphery of the Discogastrula.

The Discoblastic type of development is found in Birds, Reptiles, Teleostean and Selachian fish, in Cephalopods, some Isopods and Copepods, Scorpions, some Spiders, and a number of Flies. Probably it will be found in Monotremata and Didelphia.

The Periblastic type (Plate IX).—This is, perhaps, the most important of any one of the four types distinguished by Haeckel, for here the palingenetic phenomena are most completely disguised. Those embryonic histories which were formerly said to present no yelk-cleavage, or a superficial yelk-cleavage, belong here. The periblastic mode of development is most common in the Arthropods, in Tracheata as well as Crustacea ; it also appears from Kowalewsky's observations to occur in some Cœlenterata (Alcyonians). Like the amphiblastic and discoblastic types, the periblastic type exhibits variations, and these throw light on some of the secondary developmental phenomena in the other types. The essential point about the periblastic type is this, that the food-material collects at an early stage of development centrally, so as to be completely enveloped by the formative protoplasm. In many Arthropods this segregation of the two constituents occurs at the time of fertilisation, and we then have what has been called a blastema surrounding granular yelk. This is really the Perimonerula. Later this Perimonerula becomes a Perimorula by the conversion of its superficial 'blastema' into a number of cells. This is considered by some authorities (Weissmann, &c.) to be due in the case of the Hexapoda to the free formation of nuclei.

Good observations on a number of examples are as yet wanting, but we have the excellent researches of Edouard Van Beneden and Emile Bessels, which show that in many Crustacea the many-celled condition of the Perimorula is due to a regular cleavage process.[1]

Haeckel is inclined from this to assert as a general rule that the Perimonerula acquires a nucleus, becomes a Pericytula, and then cleaves into two, four, &c., cells, to form the Perimorula. This process is exhibited in Plate IX, in the case of a Decapod Crustacean (Peneus), studied by Haeckel at Ajaccio. It does not seem, however, at all certain that cases such as that of the Gammarus fluviatilis, described by Ed.

[1] I cannot let this notice pass through the press without alluding to the importance of Ed. Van Beneden's excellent work on the composition and signification of the egg, for the whole of the questions which turn on the presence or absence of his (deutoplasm).

Van Beneden, may not be largely diffused. In this case the separation of food-material from formative protoplasm does not take place at an early period, nor so as to produce a superficial homogeneous layer of protoplasm, covering-in a granular yelk. But the segregation takes place at separate points, so that a number of isolated cells (exactly comparable in origin to the Cephalopod's *autoplasts*) rise to the surface of the yelk and then proceed to divide, and so form a complete Perimorula. Now, it is not possible to say (in the absence of observations) how those cells arose, but it seems probable, from the analogy of the Cephalopods, that there was not a primitive division into two, and then into four, and so on, which took place deeply in amongst the granular mass of food-material, but, on the contrary, it would appear most likely that the network of protoplasm spread in and out amongst the food-granules segregated simultaneously at many points, and gave rise to autoplasts. Just as, in the Cephalopod, the segregation to form the cleavage-disc is incomplete, and leaves material behind which independently segregates at many points as autoplasts—so in *Gammarus fluviatilis*, and probably other periblastic species, the segregation to form the superficial cleavage-tunic or rind never comes off, but is reduced to a simultaneous formation of many autoplasts. In such cases the fertilised egg passes at once to the Periblastula stage, and cannot be said to exhibit either a *Peri*-monerula or *Peri*-cytula, or *Peri*-morula stage.

The food-material in the cells of the Perimorula is so placed as to occupy the inner face of each cell, and so lie centrally, but it is still disposed in the substance of the cells. By the gradual separation of the cells from it, it comes to occupy a central cavity, and is now surrounded by the cells which have freed themselves from it. This is the stage of the Periblastula (Fig. 86).

The invagination of the Periblastula to form the Perigastrula, a fundamentallyi mportant fact for the whole of the germ-layer theory, was first observed by Bobretzky in Astacus and Palæmon in 1873. It is exceedingly curious to find that in the periblastic type the invagination is so well marked, and the observation lends a certain probability to the hypothesis that invagination is the primary mode of formation of the Gastrula.

It is also exceedingly important and interesting to find that here, as elsewhere, the orifice of invagination or blastopore (Urmund of Haeckel) does not become the animal's mouth. Haeckel is not able to say, in the case of Peneus, whether it becomes the anus or not (see Plate IX, and its explanation).

A second invagination forms the true mouth and the stomodæum[1] (so-called pharynx or Vorderdarm). The hypoblastic archenteron or gastrula's stomach formed by the first invagination gives rise only to the middle portion of the alimentary canal (Mitteldarm or mesenteron) and the liver.

Here, too, as in other cases pointed out by Haeckel, the first development of the mesoblast is in the region of the blastopore-margin, but here too no certain information can be given as to the origin of all its elements, in regard to the question of their derivation from ectoderm or endoderm.

The formation of the Gastrula of the Calcareous Sponges.—Metschnikoff, Oscar Schmidt, and F. E. Schulze have recently written on the development of the Calcareous Sponges, and have shown that Haeckel's statements on this matter were not entirely correct. In the first place the formation of the Gastrula of sponges by *delamination*, and the subsequent breaking through of the mouth, has to be given up. As Haeckel observes, this is so much the better for the Gastræa-theory, for the delaminate mode of development, though, as I have pointed out, reducible to the same ultimate significance as the invaginate mode of development, is yet a cenogenesis. Haeckel doubts whether delaminate Gastrulæ really exist at all, and thinks that early invagination will be found to occur even in the Hydroids on careful study. This point should be at once investigated. Haeckel admits in reviewing the work of recent observers his fundamental error as to the mode of origin of the Gastrula of Calcareous Sponges, but rightly enough maintains that in the most essential features they have proved his statements to be justified. The observations of Metschnikoff are shown to be erroneous, as well as his conclusions by the later work of Schmidt and Schulze; in fact, he takes endoderm for ectoderm. Oscar Schmidt's observations are correct in most points, but *incomplete*, and his inferences quite erroneous, as appears from Schulze's work. Schulze confirms Haeckel in the most important point, namely, that a true gastrula with its two primary germ-layers *does* make its appearance in the development of the sponges.

The four works on the development of Calcareous Sponges furnish an exceedingly interesting series illustrative of the genesis of error in such studies. Haeckel seized upon the really great and fundamental fact that the Sponges are no Protozoa, but that, like those of Cœlenterata, their eggs give rise to a sac—the Gastrula—with a wall of two cell-layers, en-

[1] This term and its correlative 'proctodæum' I propose for the oral and anal invaginations.

doderm and ectoderm. Haeckel fell into pardonable error as to the mode of formation of this Gastrula of the Calcispongiæ. Metschnikoff, following him, had got an inkling of the true mode of formation, namely, that there was a process of invagation taking place in a blastosphere or one-cell-layered sac, but his observations were most incomplete, and his reasoning from them so much influenced by animus against Haeckel, that he has given the most erroneous account of the matter, which has been published. Oscar Schmidt failed to interpret correctly what he saw. The truth is that the Gastrula of the Calcareous Sponges, or at any rate of the species which all these observers have more especially studied, namely, *Sycandra raphanus*, is formed on the amphiblastic type. Oscar Schmidt saw the Amphiblastula (fig. 19), as did also Metschnikoff, but strangely enough, Oscar Schmidt failed to trace the invagination of this Amphiblastula to form the Amphigastrula (fig. 18), as he should have done. Consequently he mistook the whole process, declared that the Sponges had no gastrula-form at all, and concluded that all Haeckel's "schönen theoretischen Folgerungen" drawn from the fundamental fact of the Sponges possessing a Gastrula, must be abandoned.

Oscar Schmidt must have said this with some pain, for he has proved himself a very fair and well-disposed critic of the Gastræa-theory. He will therefore probably be well pleased that Franz Eilhard Schulze, working at Trieste, has made most complete observations on the egg of *Sycandra raphanus* and its development to the gastrula-form, and published them in the 'Zeitschr. fur Wissensch. Zoologie,' Bd. xxv, Supplement. Schulze has followed with absolute precision the cleavage-formation of an Amphimorula, of an Amphiblastula, and, by invagination of the large non-ciliated granular cells of the last, of an Amphigastrula in *Sycandra raphanus*. His drawings are executed with the greatest care and are obviously 'nature-true.' Thus the attacks upon the germ-layer- or Gastrula- theory from the point of view of the Calcareous Sponges are definitely put an end to. Haeckel holds, as is clearly very probable, that the Archiblastic type also occurs among Sponges, and it is possible enough that the Periblastic type also presents itself.

Summary of the facts known as to Gastrula-formation in Zoological order.[1]

Spongiæ. Archiblastic, amphiblastic.

Hydromedusæ. Archiblastic (Cassiopeia, Rhizostoma, Pelagia, *Kowalewsky*). Delaminate, probably disguised archiblastic and amphiblastic (Hydroid polyps). Amphiblastic (many Siphonophora, *Haeckel*). Discoblastic (perhaps some Siphonophora).

Ctenophora. Amphiblastic (many or all. *Kowalewsky, Fol, Agassiz*) possibly some discoblastic (*Haeckel*).

Actinozoa. Archiblastic (Actinia, Caryophyllia, Gorgonia, and Cereanthus, *Kowalewsky*, Monoxenia, *Haeckel*). Periblastic (Alcyonians, *Kowalewsky*). Delaminate, perhaps disguised archiblastic (some species of Actinia, *Kowalewsky*).

Vermes. Archiblastic (Nemertines, *Metschnikoff, Dieck*. Balanoglossus, Sagitta, *Kowalewsky*. Cucullanus, *Butschli*. Phoronis, *Kowalewsky*).

Amphiblastic (various epibolic and entobolic varieties among Platyelminthes, Nematoda, and Annelida, described by *Leuckart, Claparède, Kowalewsky*, &c., among Rotifera by *Salensky*, in the Gephyrean Phascolosoma by *Selenka*).

Discoblastic (not known, but Euaxes furnishes a transition from amphiblastic to this type, *Kowalewsky*).

Periblastic, not known.

Mollusca. Archiblastic (Argiope, Terebratula, *Kowalewsky*. Pisidium, *Lankester*).

Amphiblastic (Thecidium, *Kowalewsky*, Unio, *Rabl*, Limnæus, *Lankester* (not archiblastic), Purpura, *Selenka*, Trochus, *Haeckel*, Aplysia, Neritina, *Lankester*, Pterpods, *Fol*).

Discoblastic (Cephalopods, *Kölliker*).

Echinoderma. Archiblastic (Holothurida, *Kowalewsky*, Asterida, *Agassiz*, Echinida, *Haeckel*, Crinoida, *Wyv. Thomson*).

Amphiblastic (Uraster Mulleri, Echinaster Sarsii, Pteraster militaris, Amphiura squamata, and other viviparous Asterida, Anochanus chinensis and allied Echinida, Thelenota tremula, Phyllophorus urna, Synaptula vivipara, Cucumaria doliolum among Holothurida).

Discoblastic and Periblastic type not known.

Arthropoda. Archiblastic (some Branchiopoda and Copepoda, *Van Beneden*, Tardigrada, *Kaufmann*, the Pteromalina, *Ganin*).

Amphiblastic (some lower crustacea, *Van Beneden* and *Bessels*, some lower Tracheata).

Discoblastic (some larger forms of crustacea, *Van Beneden* and *Bessels*, Oniscus, *Bobretzky*, Scorpio (fig. 40), *Metschnikoff*).

Periblastic (most Crustacea, Limulus, *Packard*, most Myriapods, Arachnida and Insects, according to observations of *Bobretzky, Van Beneden* and *Bessels, Weismann, Kowalewsky, Claparède*, and *Metschnikoff*).

Vertebrata. Archiblastic (some Ascidians, *Kowalewsky*, Amphioxus, *Kowalewsky*).

Amphiblastic (some Ascidians, *Kowalewsky*, Cyclostoma, *Schultze*, Amphibia, Ganoids, *Owsjannikow*, Marsupials?, Placentals).

Discoblastic (Pyrosoma, *Huxley, Kowalewsky*, Selachians, *Balfour*, Teleosteans, Birds, and Monotrema?)

Periblastic. This type has not been recognised in any Vertebrata.

[1] This list and its zoological arrangement is modified and extended from the references given by Haeckel.

Note on Stenogramma Interrupta (Agardh). By E. Perceval Wright, M.D.

In the number of 'Grevillea' for December, 1874, and at p. 88, there will be found a notice, by E. M. Holmes, of *Stenogramma interrupta*, in which the author states—1. That "the tetrasporic fruit of this rare and beautiful Alga has not hitherto been recorded as occurring in Britain." 2. That it "is not described in any of the more recent works on Marine Algæ published in England." 3. That so far as the author is aware, no figure of the tetraspores has ever been published. 4. That though Miss Gifford forwarded a specimen having tetraspores to Dr. W. H. Harvey in 1848, no further notice of the plant was taken by him and that it was probably lost.

At the time when I read this notice I had fully intended to send to my friend the editor of 'Grevillea,' a reference to the 'Phycologia Australica' of Dr. Harvey, vol. iv, Plate 220, published during 1862, in which a very full account of this interesting form will be found. I omitted to do so, and I would not have thought more of the subject, but that my attention has again been called to it, partly by a communication from Mr. W. G. Farlow, assistant to Professor Asa Gray of Cambridge, U.S., and partly by a reminder from a friend to the effect that it is but an act of justice to the memory of my former master (whose post as Professor of Botany and Keeper of the Herbarium in Trinity College, Dublin, I for the present occupy), to show that he did not only receive but noticed Miss Gifford's letter. For this reason the members of the Club will be pleased to pardon the enumeration of the following to them perhaps familiar facts:

1. The "tetrasporic fruit" is described by Harvey "as occurring in British specimens" in 'Nereis Boreali-Americana,' Part ii (March, 1853). (*a*) In specimens sent to Dr. Harvey in 1848 by Miss Gifford, from Somersetshire. (*b*) In specimens sent by Mr. Isaac Carroll in 1851 from Cork Harbour.

2. It is true that Harvey's 'Nereis Boreali-Americana' was not published in England, yet it is a very accessible and most necessary work for the student of British Algæ. Harvey's 'Phycologia Australica' was however published by Lovell Reeve, at Henrietta Street, Covent Garden, London, and in

it, as well as in the North American 'Nereis,' a description of the "tetrasporic fruit" will be found.

3. On Plate ccxx, Figures 2, 3, 4, 5 of volume iv of Harvey's great work on the 'Australian Algæ,' illustrations of the tetraspores and nemathecia will be found. This is scarcely the place to criticise the former.

4. Miss Gifford's letter and specimens are in the Herbarium of Trinity College, Dublin. Her letter is dated from Minehead, Somerset, September 21 [1848]. I cannot say if it was answered, though there is proof that she was in correspondence at the time with Dr. Harvey, and that the specimens she sent were at the time registered in the Herbarium. The priority of Miss Gifford's discovery of the tetraspores is fully acknowledged in page 169, of Part ii, of the 'Nereis Boreali-Americana.'

I would further remark that Dr. Montagne's letter to the Rev. M. J. Berkeley is alluded to by Dr. Harvey in the work last quoted; also that J. G. Agardh's 'Species Genera et Ordines Floridearum,' which forms the second volume of the 'Species Genera et Ordines Algarum,' was published not only in parts but in fasciculi, and that vol. ii, pars. ii, 1, pp. 1 to 504, containing the description of Stenogramma, was published in 1851. (Pars. ii, 2, was published in 1852.) As this fasciculus was probably printed early in 1851, it is not likely that Agardh could have seen Dr. Montagne's letter in the June number for that year of the 'Annals and Magazine of Natural History.'

The Trinity College Herbarium possesses specimens from the following localities:

Europe.—Strangford Lough; in six to eight fathoms, adhering to small stones in Castle Ward Bay, between Portaferry and the Old Castle, Dr. Dickie, 1858. Cork Harbour, Isaac Carroll, 1851. Minehead, Somerset, Miss Gifford, 1847-48. Plymouth, Dr. Cocks, 1846; Rev. W. Hore, 1847; Mr. Gilbert Sanders 1847. Lisbon, "In Tago salso, lecta mense September, 1842, F. Welwitsch." Gibraltar, Mrs. Craige. Toulon, M. Giraudry.

America.—Florida, Key West, Dr. Harvey. California, San Francisco, Dr. Sinclair.

Australasia.—New Zealand, Colenso. Tasmania, F. Mueller; W. H. Harvey.

In many specimens, especially in luxuriant ones from New Zealand, with fronds over a foot in length, the nemathecia are distinctly to be seen on both surfaces of the frond; but the shape of the nemathecia varies much, and so do the terminal edges of the laciniæ, being sometimes broadly obtuse

and at other times almost pointed; often the edges of the frond are "entire," but sometimes they are clothed with dense tufts and fringes of "proliferous forked leaflets."

The specimen with tetraspores first examined by E. M. Holmes was from Mr. Isaac Carroll's collection, and was doubtless gathered in Cork Harbour. The one from which the figure on Plate xxxvii of Grevillea was drawn is stated to have been gathered in Scotland. It would be interesting to know from what part, as this will be the most northern habitat as yet known.—*Extract from Minutes of Dublin Microscopical Club for November* 18, 1875.

Preparation *of* Sections *of* Cerebral *and* Cerebellar Cortex *for* Microscopic Examination. By W. Bevan Lewis.

The histological analysis of animal tissues, so far as it relates to processes of preparation and staining for the better differentiation of the elementary constituents under microscopic examination, has long been regarded as an important item in physiological and pathological research. Much has already been accomplished in this sphere of labour, yet much still remains to be done, especially in the histology of the central nervous system. New statements in regard to the staining of brain sections which have recently been advanced require mature consideration and experimental verification before they become standard facts for the guidance of the physiological inquirer. Until this sifting of evidence is accomplished we cannot hope to find any substantial basis for the establishment of histological science.

The constituents of the cerebral cortex differ much *inter se* in the facility with which they acquire the coloration necessary for a good delineation of their outline and structure. Thus, while it is but a trivial task to stain equably and deeply the smaller cells of the different layers of a convolution or the granular layer of the cerebellum, or even to bring distinctly into view the nuclei of the blood-vessels or the contour of the nerve-fibres, it is a far more difficult matter to effect a good staining of the pyramidal cells or the process of the cells of Purkinje. By a *good* staining I mean a *deep* staining, which yet leaves the cell contents as unaltered as possible, the nucleus and nucleoli clearly exposed to view, and the numerous minute extensions of these bodies well defined. Undoubtedly a really good section

of the cortical layer of the brain should embrace all these qualities, together with a fair view of the vascular channels, whilst the delicate reticular arrangement of the surrounding matrix should be unaltered by the reagents employed. The difficulties in our way are great, but yet of late patient and laborious investigation has rewarded the inquirer with frequent triumphs of a most substantial character. Of the numerous colouring agents used by the microscopist, the more valuable for purposes of studying cerebral histology are the following: carmine, logwood, picric acid, aniline blue, aniline black, magenta or rosaniline, aniline red or fuchsin, and the various Judson's dyes. We owe important assistance also to the reduction of certain metallic compounds by the agency of the organic germinal centres of the tissues, *e.g.*, osmic acid, chlorides of gold and palladium, oxide of uranium and nitrate of silver. The latter series I shall not refer to further in this article, as my attention is to be given entirely to the members of the first series, which give their own natural colour to the objects exposed to their action. I may, however, in passing, recommend solutions of each of the latter class of the ordinary strength of one per cent. to be kept in hand.

For actual use the solutions of gold and silver may then be diluted to the extent of 0·25 to 0·5 per cent., the chloride of palladium to 0·1 per cent., the chlorides of gold and potassium combined should represent in every 100 cc. of the solution $\frac{1}{100}$th of a gramme of the salt, whilst the osmic acid solution should vary from 1 to 2 per cent. in strength.

With this brief mention of the metallic dyes I pass on to the subject more immediately concerned and describe *seriatim* the several processes employed by myself for staining the cortex of the cerebrum and cerebellum. First in the series is carmine, the earliest agent used for this purpose.

Carmine dyeing and its modifications.—Gerlach, with whom originated the process of dyeing, used a solution of carmine which has since been modified by Thiersch, Beale, and Frey. The solution recommended by Beale (a carminate of ammonia) is, I believe, the one most generally adopted, and possesses, with some drawbacks, several points of advantage over other media. The whole process of carmine-staining demands, however, great attention to details to secure perfect success. One essential feature is that the solution should contain but a minimum amount of ammonia, and this is of special importance when we are dealing with cerebral tissue. Again, the after process of washing should be conducted

with a very weak acid solution, the best wash being a half to one per cent. solution of glacial acetic acid. Small portions of the cortex, a third of an inch in thickness, are previously hardened in the bichromate of potash or in Muller's fluid, using chromic acid solutions as accessories to hurry on the process near its completion, are then transferred to Stirling's microtome and the finest possible sections made. They should now be placed for a few minutes in methylated spirit, and next passed into Beale's carmine solution, diluted with seven times its bulk of distilled water. I find my sections are usually stained to the requisite depth of colour in six or eight hours, but variations in rapidity will constantly occur, the delay being frequently occasioned by the presence of free chromic acid, which should always be completely removed by immersion in spirit prior to section cutting; and we can readily conceive with Beale that any changes undergone by commencing putrefaction in the nuclei will, by altering the acid reaction which is the natural post-mortem condition, interfere with the necessary decomposition of the carminate. Sections that have assumed the proper tint may now be removed to a capsule containing a half per cent. solution of glacial acetic acid. They are next washed from all acid, dehydrated by steeping in absolute alcohol or rectified spirit, cleared with oil of cloves or anise, and mounted in balsam. This is the usual method adopted by me in single staining with carmine, but the results, though good, are by no means so uniformly satisfactory as by log-wood and aniline processes. There is one measure, however, which I adopt with this reagent with the best results; I may denominate my process as a mode of differentiation by peculiar alterations in the refractive indices of the structural elements. Changes which occur to the cell-contents and their processes —I refer to alterations in density by the agency of *heat*, *spirit*, *coagulating media*, and the *essential oils*—alter the refractive relationships previously held by them to the surrounding matrix, and thus bring into view what would otherwise be passed by unnoticed. It appears to me that this variety of histological analysis may, in regard to brain sections, be turned to very good account. On placing an unstained section of cerebrum or cerebellum in the field of the microscope, saturated with spirit, little or no structure is apparent, but if a drop of essential oil be now allowed to run over it there will be observed at a certain stage of the clearing up, and whilst the spirit is evaporating, a sudden starting out in bold relief of the cells, nerve-fibres, vessels, &c., which again disappear or partially fade on perfect clearing

of the section. Now this appearance may be fixed by suddenly dropping over its surface a little balsam and permanently mounting. Upon this fact depends the process now to be described. Sections treated by Beale's carmine solution (1 to 7 in strength) and washed with the acid wash are placed, saturated with spirit, upon a slide. When the spirit has nearly all evaporated a drop of oil of anise is allowed to flow *over* the section (not to float it up), and the clearing process is watched on the stage of the microscope; then, just when the appearance referred to above is presented to view, a drop of balsam is allowed to run over the section and a covering glass permanently fixed on. In lieu of the oil of anise I frequently employed glycerine with the same results, and mount ultimately in glycerine jelly. Still better effects may be obtained by the following method.

Picro-carmine Staining.—I use the solution recommended by Ranvier, the sections being left in it for an hour. I then transfer them to a strong ammoniacal solution of carmine for the space of twenty minutes, and on removal wash freely and mount, as in the process described above. The appearance of sections of cerebellum treated thus is very striking, and the cells of Purkinje and their processes are far better delineated than by the usual carmine method.

Logwood Staining.—Formulæ for the carmine dyes are well known; not so, however, those for hæmatoxylin. Böhmer, who first employed logwood for these purposes, gives a formula which is quoted by Frey.[1] I may also refer the reader for an excellent formula for the logwood solution to the pages of 'The Lens' for July, 1872. The solution usually sold under this name varies greatly in efficiency, and I am told by one of the most eminent authorities in cerebral histology that this variability has proved a matter of great and constant annoyance to him. I must confess to a similar experience in my employment of this reagent. When, however, the proper solution can be obtained, it undoubtedly stands in the foremost ranks of staining fluids, its results being unrivalled for the major details in the cerebral cortex. Of the solution sold as logwood dye about ten drops should be added to each drachm of distilled water, carefully filtered and exposed for a short period to the air. The sections should remain in it until the required depth of colour is obtained, the logwood poured off, and the pieces well washed by allowing a stream of water to flow on to the inclined side of the porcelain vessels in which they are held. All diffuse

[1] 'The Microscope and Microscopic Technology,' translated by Cutter, page 158.

staining may now be removed, by keeping them in a vessel containing methylated spirit for some hours. When sufficiently washed by this measure, as may be seen immediately by the removal of a section to the stage of the microscope, and examining it with a low power, the tint of the germinal centres dyed in this way may be darkened by exposure of the slide for a few seconds to the vapour of ammonia, or to a faintly alkaline spirit. They are next cleared in the usual way with oil of cloves, and mounted in balsam.

The Aniline series of Dyes.—Magenta or rosaniline is a powerful and active dye, but its solubility in spirit is so great that its effects are not permanent, and the difficulties in fixing this reagent have proved insuperable obstacles in the way of its employment for these purposes. Judson's purple dye also shares in this disadvantage, but to a less extent. The latter may be used in producing the peculiar conditions of refractive power in the tissue elements which are described under the heading of carmine; but for these results the picro-carmine solution far surpasses any of the aniline or Judson's series of dyes. To Mr. W. H. O. Sanky belongs the credit of introducing to our notice the aniline black as a staining reagent applicable to the microscopic investigation of cerebral tissues. A section of human cerebellum exhibited by him at the last annual conversazione, held at the West Riding Asylum, attracted great and deserved attention. The cells of Purkinje and their processes were shown with extraordinary beauty. His process has been described in the pages of the 'Lancet;' but for full details I would refer my reader to the 'West Riding Asylum Reports' for this year, where an extremely interesting paper by Mr. Sanky will be found, together with most faithful and beautiful plates of the cerebellar cortex, &c. The process he adopts for *drying* fresh brain for sections I have not tried, but the aniline black as a dye I have used extensively and with the best results. My experience leads me to recommend two distinct methods as productive of most satisfactory slides. The solutions of aniline black employed by me are three, varying in strength as follows: 0·25, 0·5 and 1 per cent. It may be dissolved in water, alcohol, or even glycerine; but for most purposes the aqueous solution is the most useful. It is a matter of no difficulty with this reagent to dye to any depth, but the washing-out of diffuse staining is at first rather perplexing. The solvents of aniline which present themselves as useful accessories here are absolute alcohol, methylated or rectified spirit, glycerine, or glycerine and water. These, however, do not not succeed with me in demonstrating satis-

factorily the processes of the cells of Purkinje, or the finest of twigs which arise from the pyramidal layers of the cerebral cortex. No reagent has found such claims upon our attention for this purpose as chloral hydrate. For some time past I have employed this reagent for the demonstration of nervous elements, having been directed to its use by a paper by Victor Butzke.[1] This writer states:—"Eine solution von chloralhydrat in wasser, von 1 : 1 bis 1 : 10 pro cent. auf das frische Gehern angewandt, löst zwar das Hirnfett nur theilweise, versetzt es aber in einen Zustand, in welchem es molecular zerfällt, so das es leicht aus dem Zusammen hange mit den Formelementen herausgewaschen werden kann. Als ganz vorzüglich habe ich eine combination von Hyperosmiumsäure ($\frac{1}{4}$ per cent.) mit chloralhydrat gefunden, was nach einander angewandt, in der Schönheit der Isolation der Hirnelemente bald, so glaube, ich die alten Rivalen weit hinter sich lassen wird." The latter statement I have not been able at present to confirm; but as to the full value of chloral hydrate for such purposes I can fully confirm the opinion of Butzke. Still another important quality belongs to this compound, namely, that it is a powerful solvent of aniline black, so that we possess in it the very valuable combination of qualities most likely to give us the best results. This assumption, put practically to the test, has answered my highest expectations. The process I employ I can confidently recommend, especially for the demonstration of the cerebellar cortex. The sections are first deeply stained in aniline dye, gently washed from superfluous colour in distilled water, and removed to a porcelain vessel containing the solution of chloral, where they are allowed to remain from twenty to thirty minutes. They are next transferred to the following solution:—Of solution of chloral hydrate and oil of cloves equal parts. Alcohol enough to dissolve perfectly and form a perfectly clear solution. The alcohol must be added by degrees, the mixture being stirred with a glass rod, and care being taken to avoid any excess. During use this mixture must of course be kept carefully covered up to avoid evaporation. It will now be found that the sections are clearing up, whilst all diffuse staining is still further removed by the chloral. We should remove one occasionally to a slide, and examine under a low power the progress made, and when satisfactory results are obtained, the sections should be washed with a little alcohol, floated up by one or two drops of clove oil, and when perfectly clear mounted in balsam. In regard to the essential oil used I

[1] 'Archiv für Pyschiatrie und Nervenkrankheiten,' 1872.

employ oil of anise and oil of cloves indiscriminately, both being of high refractive powers. The former has, however, the disadvantage of solidifying at ordinary temperatures. Its oxidized compound stearoptene, which forms the greater bulk of the oil, and which separates in a solid mass, may, however, by a *very* gentle warmth be kept in a fluid state during the process. The beautiful effects of logwood staining may also be obtained for the granular layer, whilst the layer of Purkinje's cells and their antler-like processes may be produced with all the accurate details of aniline-staining by a process which I now proceed to describe.

Double Staining with Logwood and Aniline.—It will soon be noticed by those who attempt a logwood staining of the cerebellar cortex, that whilst the granular layer nuclei of blood-vessels and nerve-fibres are beautifully stained, the cells of Purkinje and their processes remain very faintly or not at all affected by the colouring reagent; the aniline black, on the other hand, appears to possess special affinities for these latter structures. If, therefore, sections dyed with logwood are immersed for *a few seconds only* in a solution of aniline black of a strength of 0·25 to 0·5 per cent., it will be found that the logwood staining still remains, whilst the layer of Purkinje has taken up the aniline sufficiently to afford a decided and beautiful contrast. The chloral hydrate should now be used, but the alcoholic solution is not here advisable. They should be well washed from the chloral with a stream of water, *rapidly* dehydrated by alcohol, cleared with oil of cloves, and mounted in balsam.

Summary.—Having so far dwelt on the more general methods employed for demonstrating the structure of the cineritious substance of the brain, I shall in conclusion briefly sum up their special advantages and disadvantages in individual cases. First, in regard to carmine stainings, there are two great faults which all microscopists will, I think, immediately recognise. One defect is the glare of a colour such as carmine, which is very tiring to the eye, and renders prolonged and steady working with the microscope a matter of great difficulty, not to say real injury, to the eye. Then, again, the want of definition given by a deeper, darker colour is felt by all experienced microscopists, and more especially by those who have employed the logwood and aniline dyes. On the other hand, it never clouds the cell, but leaves the nuclei and all minor details clearly exhibited. No one who has examined a really good preparation of the cerebral cortex with logwood staining will fail to acknowledge the great superiority of this dye over carmine

in its general results. As regards the peculiar refractive qualities, however, which are impressed upon the tissue elements by the modified carmine process above described, I may state my belief that this will yet prove of most essential service in the estimation of the relative proportions of cell-processes in any individual section, and the most accurate tracing of any existing connections, for not by the deepest aniline staming have I yet succeeded in demonstrating the existence of so thick and numerous a series of processes diverging from the pyramidal layers of the cerebral cortex as by the methods described above. With regard to the smaller cells of the cerebrum and cerebellum the logwood staining is eminently successful; but when it is our object to bring into view the larger cells and their extensions, I would recommend the aniline dyeing above described, or the double staining with logwood and aniline.

On the Evolution *of* Hæmoglobin. By H. C. Sorby, F.R.S., F.L.S., F.Z.S., President of the Royal Microscopical Society.

In a paper read before the Royal Microscopical Society last April, published in the 'Monthly Microscopical Journal' for May,[1] I endeavoured to show the very great importance of referring all the measurements of spectra to the wave-lengths of each part expressed in millionths of a millimeter; and in another paper read before the same Society in November, published in the above-named journal for December,[2] I described a new form of apparatus by means of which the wave-lengths can easily be measured to a millionth of a millimeter, and the difference in the wave-lengths of the centre of the bands in closely related spectra determined with still greater accuracy. In the former paper I also endeavoured to point out the very important conclusions which may be drawn from those small differences in the position of the absorption-bands, which might easily be overlooked, and which in some cases I had myself overlooked until quite recently. Unfortunately the full meaning of some of these differences cannot yet be ascertained with

[1] Vol. xiii, p. 198.
[2] Vol. xiv, p. 269.

perfect certainty. The physical facts have altogether outstripped chemical knowledge. They clearly point out what kind of investigation is necessary, but at the same time it would be very difficult, if not impossible, to obtain a sufficient quantity of material to decide some of the most important questions. In the present state of the subject the only course open to us is to draw the most probable analytical conclusions that we can by careful induction from facts already determined by synthetical methods.

The best illustration of this kind that I can give is the difference in the spectra of hæmoglobin, in which the oxygen is replaced by other gasses without there being any actual decomposition. Thus, for example, on replacing the oxygen by nitric oxide, or by carbonic oxide, the general character of the spectrum of oxidized hæmoglobin is only slightly changed, but the position of the bands is materially different. When expressed by wave-lengths in millionths of a millimeter, the centres of the absorption bands are as follows:[1]

Nitric oxide hæmoglobin	583	545
Oxygen	581	545
Carbonic oxide	575½	542

It will thus be seen that the substitution of one gas for another in loose combination with the same very complex coloured radical hæmoglobin alters the wave-lengths of the centres of the bands to an extent which can be easily measured. This change in the spectra is accompanied by most decided differences in chemical characters, so decided in fact that, as is well known, the substitution of the oxygen in the blood by carbonic oxide makes all the difference between life and death. In all these cases, however, it is easy to prove that there is one complex radical common to all, since they can all be changed into identical products. Now, supposing that these three substances were met with in nature, and that their spectra were known, and also the fact of their yielding common products, it is quite clear that we should be justified in concluding that they were closely related in containing a common radical, and at the same time differed by the substitution of one substance combined with this radical by another substance. I think that the strict accuracy of this reasoning cannot be denied in this

[1] Since the wave-length method has not yet been adopted by all observers, it may be well to give the wave-lengths of a few of the Fraunhofer lines that occur near the absorption bands described in this paper.

D. 589. E. 527. F. 486.

instance; and in applying it to less known facts, I think I may claim high probability in favour of the provisional conclusions. It would indeed be premature to look upon the results as more than provisional, seeing that the whole subject is in its infancy.

At the present time it would be impossible to give a more striking illustration of this method of research than the various facts which seem to indicate the gradual evolution of hæmoglobin. Very much remains to be learned, and I now publish what I have so far been able to learn, chiefly with the view of attracting attention to a most promising field for research, and to show the importance of the spectrum method in the investigation of the comparative physiology of the lower animals.

Some few months ago I commenced the study of the different coloured substances found in the shells and soft parts of various species of Mollusca. Many of these have very striking characters, but on the present occasion I shall confine my remarks almost entirely to those which bear on the evolution of hæmoglobin.

If the common large snail, *Helix aspersa*, kept some time without food in autumn, be killed with chloroform and then immediately dissected, it will be found that the intestine near to the part where the secretion from the liver passes into it, is more or less distended by a reddish-brown liquid, which easily runs out when the intestine is cut. There cannot, I think, be any doubt that this bile is a mixture of at least two coloured substances. One is probably related to the brown substance, quite unlike the chief coloured constituent of the bile of the higher vertebrata, which is the cause of the dark colour of the liver, especially in *Helix nemoralis*, and gives no well-marked or characteristic spectrum. The other gives one very dark and well-defined absorption-band towards the yellow end of the green, and another much more faint towards the blue end of the green. That this substance is not a product of decomposition is proved by the fact that the bands can be well seen in light transmitted through a living *Limax*. At first sight the spectrum might easily be mistaken for that of deoxidized hæmatin, but on comparing them together side by side, though the bands of this bile pigment are of exactly the same character as those of the deoxidized hæmatin from human blood, they are in a most decidedly different position. There is, in fact, the same kind of relation between these two spectra as that between the spectra of hæmoglobin when loosely combined with different gases. This bile pigment,

however, differs from normal deoxidized hæmatin in not uniting with loosely combined oxygen when exposed to the air. In order to raise it to an oxidized state, it is necessary to add a minute quantity of potassic permanganate. The well-marked absorption bands then disappear, but are again developed by the addition of a deoxidizing reagent. This spectrum is, however, not seen unless the solution be somewhat alkaline; and when the natural bile is exposed some time to the air, so as to become somewhat acid, the bands may not be seen until a slight excess of ammonia has been added. Hence clearly enough, as in the case of normal hæmatin, the above-named bands in the spectrum are characteristic of the alkaline deoxidized solution, but this bile pigment of *Helix* has a less affinity for loosely combined oxygen. Such then being the case, it is necessary to inquire whether it be possible to change it into normal hæmatin, or to alter both into one common product.

As far as I have yet been able to ascertain, there are at least three very distinctly different modifications of hæmatin, which, when in an alkaline solution and deoxidized, give spectra of almost exactly the same character, the bands are at the same distance apart, but their centres are situated in very materially different wave-length positions, as will be seen from the following table:—

	Very dark band.		Much fainter band.
Bile pigment hæmatin	564½	. .	532
Fresh made normal hæmatin from human blood	561½	. .	529
The same kept several years sealed up in a tube	556¾	. .	524¼

On taking a small quantity of an aqueous solution of human blood, adding ammonia, and boiling, we obtain a brown solution of what I regard as normal hæmatin. Adding a little potassic sodic double tartrate, and deoxidizing with a minute portion of ammonium ferrous sulphate, we obtain the well-known spectrum of deoxidized hæmatin. If, however, the boiled ammonical solution of blood be sealed up in a glass tube and kept for several years, it becomes deoxidized by its own decomposition, and the spectrum, though very closely analogous, is not identical with that obtained on deoxidizing the fresh made solution. Both bands are raised towards the blue end to the extent of nearly five millionths of a millemeter, the upper one being also made somewhat broader. I may here say that these quantities are as distinct and easily measured with suitable apparatus as an equal number of inches in the height of a man.

According to the principle already named, such a relation between the spectra indicates some definite change of the

hæmatin into a closely related product. Much light is thrown on this question by the action of potassic permanganate. On adding a small quantity to normal hæmatin formed by boiling human blood with dilute ammonia, it is first changed into a modification which, when deoxidized, gives similar bands in exactly the same situation as those in the solution kept long sealed up in a tube; but yet there is such a decided difference in their width as to make it probable that the two products are not exactly the same substance. The facts do, however, prove that normal hæmatin can be changed by slight oxidization into an analogous substance, giving the absorption bands somewhat nearer the blue end of the spectrum. On adding still more permanganate this is still further and very completely changed into an entirely new product, which when deoxidized gives a well-marked band in the yellow, having its centre at wave length 587.

Now, on treating the hæmatin of the bile of *Helix* with potassium permanganate, it is in the first instance so changed as to give on deoxidization exactly the same spectrum as normal hæmatin, with the bands in exactly the same position; and further action of the permanganate alters it into the other modification already named. I have not, however, succeeded in preparing the product giving the band in the yellow, probably on account of the presence of so much of more easily oxidized substances, which make the bile so thick and gummy. It thus appears almost certain that the hæmatin of the bile of *Helix* can be changed into normal hæmatin by a process of oxidization, and that this change modifies both the spectrum and the affinity for loosely combined oxygen. We may, therefore, very reasonably expect to meet with this normal modification in some of the organs of the living animals, and, in fact, I think it very probably does occur in the muscles of the foot and elsewhere, since the absorption band is certainly nearer to the blue end and nearly corresponds with that of normal hæmatin. I have, however, not yet proved this to my entire satisfaction, for want of proper material and good direct sunlight since I perceived the importance of the question.

The amount of hæmatin found in different species and genera of pulmoniferous molluscs differs very much. In *Testicella*, *Pupa*, and *Clausilia*, there was so little as to make its presence very doubtful. The amount in *Limnæa* is small, but yet very decided, and is much greater in *Helix*, *Zonites*, *Limax*, and *Cyclostoma*. In all these its presence is usually most decided in the liver, but yet it is not confined to that organ. I have not been able to detect it in the blood itself,

but if it is really formed in the liver, it seems probable that it must to some small extent pass into the blood, or else it could scarcely find its way into the other organs. I have also met with it in *Planorbis corneus*, in the liver, and in another glandular organ with a granular structure; but the most important coloured substance in the animal is a modification of hæmoglobin dissolved in the blood. This compound is of such a remarkable character as to claim very special attention.

In its natural state this red blood of *Planorbis* gives so nearly the same spectrum as that of the hæmoglobin of human blood, that it was regarded as the same substance by Ray Lankester.[1] There is, in fact, no difference in *general character*, and, like the normal kind, when deoxidized, the two bands disappear and are replaced by a broad band; but yet on careful comparison it may easily be seen that the two well-marked bands of the oxidized state lie nearer to the blue end than in the spectrum of normal hæmoglobin, as will be seen from the following table:—

	Centres of the bands.	
Normal hæmoglobin	581 .	545
Planorbis	578 .	542½

Even if there be a little doubt as to the exact wave-lengths true to a millionth of a millemeter, there is none as to the fact of the bands being nearer to the blue end by a wave-length of 2½ or 3 millionths. This difference cannot be due to any difference in the physical state of the substance, since in both cases it was perfectly dissolved in a relatively large quantity of water. It is rather of such a character as would result from the substitution of one kind of albumenoid for another in combination with the same modification of hæmatin, into which both are decomposed when boiled with ammonia or decomposed by acids. This conclusion is made almost certain by the difference in the temperature at which they are decomposd by the coagulation of the albumenoid. The red aqueous solution of Planorbis hæmoglobin slowly turns brown, and is decomposed into hæmatin at the comparatively low temperature of 45° C., and is rapidly changed and coagulated at 49°; whereas in the case of a similar solution of human hæmoglobin, similar changes do not occur until the temperature is raised to 65° and 69°. There is thus a difference of about 20° C. in the temperature at which the albumenoids coagulate; and this fact taken in conjunction with the character of the spectra appears to me very satisfactory evidence of a well-marked difference in the albumenous con-

[1] 'Proc. of Roy. Soc.,' vol. xxi, p. 70.

stituent of these two modifications of hæmoglobin. If this be so, we might reasonably expect to find some difference in their chemical relationships, whilst at the same time both might yield identical coloured products. This is most certainly the case. When treated with citric acid both yield the same hæmatin, but the hæmoglobin of human blood is far less stable and far more easily decomposed than that from *Planorbis*. Taking an equal quantity of both, and adding an equally small quantity of citric acid, the hæmoglobin of human blood was changed almost at once into hæmatin, whereas that from *Planorbis* showed no such sudden decomposition; and even after half an hour was not so much changed as the other in less than a minute. In another experiment, taking equal quantities of the two hæmoglobins, I added twice as much citric acid to the *Planorbis*. That of human blood was changed to hæmatin and lost the hæmoglobin bands in ten minutes, whereas that from *Planorbis* was changed so much more slowly that the alteration was not complete until after two hours, though, as I have said, the amount of citric acid was double. Hence clearly enough the hæmoglobin of *Planorbis* is a far more stable compound. I am also much inclined to believe that some of the secondary spectra do also differ in important characters. Thus, when the hæmoglobin of *Planorbis* was deoxidized, I could see a faint absorption band at about wave-length 504, not visible in the case of deoxidized human hæmoglobin. This may be due to some other colouring matter mixed with the original, but the spectrum of the oxidized compound furnishes no evidence of any such second substance. Taking everything into consideration, it appears to me that the complete study of these two modifications of hæmoglobin cannot fail to throw very much light on a number of interesting physical and biological questions. It also appears to me very desirable that the hæmoglobin of the various animals in which it has been detected by Ray Lankester and others should be carefully examined from this new point of view, in order to ascertain whether the two modifications are correlated to the existence or non-existence of blood discs, since perhaps it may be found that the modification of globulin met with in *Planorbis* is characteristic of blood coloured by a hæmoglobin *solution*, and the other modification that which is essential to the formation of coloured blood *discs* in a nearly colourless serum.

According to the observations of Ray Lankester,[1] there is strong reason to believe that hæmatin is the coloured radical of his *chlorocruorin*, which plays the part of hæmoglobin in

[1] 'Journal of Anatomy and Physiology,' vol. iv, p. 125.

the green blood of *Sabella ventilabrum*, though the exact connection remains to be determined.

Reviewing now the whole subject from the point of view of the theory of evolution, it should appear that the most stable form of hæmatin made its appearance as a bile pigment in a state totally unfit for the purposes of respiration, since it will not unite with loosely combined oxygen on exposure to the air. By a slight change in its constitution it might however perform this function, and perhaps to some extent does in some of the Mollusca, or at all events supplies the place of the hæmoglobin which forms one of the constituents of the red muscles of higher animals, whilst in some of the Gasteropoda it is so combined as to give rise to a form of hæmoglobin. According to Lankester,[1] *Planorbis* alone amongst this group of animals has a red blood, coloured by hæmoglobin, perhaps, as he suggests, because the necessity for utilizing the whole of the small amount of oxygen available in the water of stagnant marshes, rendered the further development of the respiratory fluid very advantageous. This was brought about by the modified bile hæmatin combining with an albumenoid, so as to give rise to the modification of hæmoglobin found in the blood, which is certainly a more suitable oxygen carrier than hæmatin, since it can be deoxidized and reoxidized without decomposition. Advancing to higher animals, it appears to have been very advantageous to have this oxygen carrier, not in solution, but in the form of discs suspended in a nearly colourless serum, so that these red discs might be filtered out, and it alone penetrate to some parts. This was perhaps brought about or accompanied by the substitution of the original albumenoid by another, whilst at the same time hæmatin ceased to be formed as a secretion of the liver. The green blood of *Sabella* perhaps resulted from an analogous development in another way, not yet fully understood. In all these changes, as we advance from the most rudimentary condition, we find that, though the fundamental coloured radical, which determines the oxygen-carrying properties, remains the same, the compound becomes more and more unstable, and is more and more easily decomposed, which is in all probability correlated to a greater and greater vital power, capable of counteracting stronger and stronger chemical affinities.

This advance from a very stable to a very unstable compound, is in many respects analogous to what I have found to occur during the growth of orange-coloured flowers exposed to varying light. When developed nearly in the dark the

[1] 'Proc. of Roy. Soc.,' vol. xxi, p. 78.

petals are coloured mainly by the more stable compounds which absord only the blue end of the spectrum, whereas when developed under the the influence of bright sunlight other compounds are formed, which absorb light more towards the red end of the spectrum, and are much more readily decomposed. By exposing to the light the mixed solution of the colouring matters developed in the living plant under the influence of light, these less stable substances are soon destroyed, and we obtain a mixture closely corresponding to that met with in those petals which are developed in the dark. All this may be explained by supposing that the increased vitality due to the exposure of the plant to the sunlight suffices to counterbalance a much greater amount of mere chemical affinity.

Such then is the best account I am now able to give of what appears to me to be a most promising field for research. Of course I cannot but feel that it is very imperfect, but yet I trust that it will suffice to show the great value of this method of research in the study of comparative physiology, and the importance of attending to minute differences in spectra, since this may often lead to the discovery of very striking chemical differences which otherwise would never have been suspected. It is much to be regretted that the amount of material usually available will make it very difficult, if not impossible, to confirm such results by accurate chemical analyses, but at the same time this makes the value of the spectrum method still more apparent, since it is possible by that means to learn a large number of most important facts, which in all probability would for ever have escaped detection by pure chemical analysis. Now that the gradual evolution of complex chemical compounds essential for the performance of physiological processes has been forced on my attention, I can clearly see that this view of the subject will throw much light on other cases; and it appears to me that for the future we must not confine our attention to evolution of mere form, but materially extend the scope of our inquiry, with the hope of being thereby able to better understand the manner in which has been brought about the wonderful correlation between structure and function.

P.S.—Since writing the above my attention has been called to the following remarks made by Ray Lankester in his paper in 'Pflüger's Archives.'[1] He there says—"The

[1] "Ueber das Vorkommen von Hæmoglobin in den Muskeln der Mollusken, und die Verbreitung desselben in den lebendigen Organismen," 'Pflüger's Archiv für Physiologie,' Bd. iv, s. 318.

chemical differences of different species and genera of animals and plants are certainly as significant for the history of their origin as the differences of form. If we could clearly grasp the difference of the molecular constitution and activities of different kinds of organisms, we should be able to form a clearer and better grounded judgment on the question how they have been developed one from the other than we now can from morphological considerations." As will be seen, this view of the question agrees remarkably with my own, though he was led to it by more general considerations, before such a striking illustration of the principle had been discovered as almost to compel us to admit that there must have been a gradual evolution of such important and complex chemical compounds as hæmoglobin, a substance which contains the same constituent radical in the various stages of its development.

REVIEWS.

The Anatomy of the Lymphatic System. By E. KLEIN, M.D., Assistant Professor at the Laboratory of the Brown Institution, London. II.—*The Lung.* London: Smith, Elder, & Co., 1875.

IN this handsome volume Dr. Klein gives a full account of his researches into the minute anatomy of the lymphatics of the lung and pleura, a short summary of which had already been communicated by him to the Royal Society.[1]

The work is divided into two sections, the normal conditions being described very fully in the first, while the second is occupied by a most interesting account of the pathological changes in acute and chronic inflammation, in the artificial tuberculosis of guinea-pigs, and in the acute miliary tuberculosis of man.

Commencing in the first section with the pleura, Dr. Klein points out a remarkable difference in the appearance of its endothelium in the distended and collapsed lung. In the former, in which it has to cover a wider area, the endothelium is seen as flattened plates, rather thicker in the centre, with a flattened circular nucleus, and only faintly granular body; whereas in the collapsed lung the cells are distinctly granular, and are moreover no longer flattened, but shortly columnar, with a spherical nucleus. The tops of the cells are seen to be rounded, leaving a considerable space between neighbouring cells, the deeper portions only of which are cemented together. The endothelium of the costal pleura consists of flattened plates, so that it differs from that of the pulmonary pleura in the same manner as Waldeyer has shown that of the surrounding part of the peritoneum to differ from that of the upper part of the ovary, the cells in each case bearing a close resemblance to an epithelium. Passing on to the matrix of the pleura, Dr. Klein describes it as consisting of extremely delicate connective tissue with a few elastic

[1] 'Proc. Roy. Soc.,' January, 1874.

fibres, small spaces occupied by connective tissue corpuscles, and communicating more or less completely with each other, being left between the bundles, and representing the lymph-canalicular system. In guinea-pigs a meshwork of unstriped muscle-fibre was also found, more especially developed in those parts which move most freely in respiration, and showing in the meshes lymphatic lacunæ, which communicate with the rich subpleural lymphatic plexus. The vessels forming this arise in the superficial alveoli, receive branches from the deeper parts of the lung, and discharge themselves into trunks that run in the ligamenta pulmonis to the bronchial glands. Dr. Klein has satisfied himself of the existence of stomata forming a communication between the cavity of the pleura and the above-mentioned superficial lymphatic plexus and intermuscular lymph-spaces; so that when these stomata are dilated (as happens in inspiration) the lymphatic system of the lung may become filled with whatever matter may occupy the pleural cavity. The stomata are best seen in the lungs of animals suffering from chronic pleurisy, when their position becomes very clearly indicated by a germination of the endothelium at their margins.

Dr. Klein next describes the lymphatic system of the bronchi. This consists of a rich network in the adventitia, constituting the peribronchial lymphatics, which receive branches from the submucous tissue, and anastomose with the perivascular lymphatics which accompany the blood-vessels. In the guinea-pig's lung (especially in animals suffering from artificial tuberculosis) there are spherical, oblong, or even cord-like accumulations of adenoid tissue in the wall of many peribronchial lymphatics. The larger ones are provided with a special network of capillary blood-vessels. These were suspected by Burdon Sanderson, who first described them, to be connected with the lymphatics, and are shown by Dr. Klein to be what he has called "peri-lymphangeal follicles," consisting of adenoid tissue in direct connection with the lymphatic wall. These follicles were also found in the rabbit's lung, but less numerous and not of so dense a structure. The rootlets of the peribronchial lymphatics consist chiefly of a system of communicating spaces in the mucosa, the muscularis, and the submucosa, which are interfascicular, and vary in size according to the amount of separation of the contiguous bundles. They are smallest in the mucosa, where they consist of lacunæ and anastomosing canals, whereas in the adventitia they are elongated or rhombic spaces; the former spaces are occupied by branched connective-tissue-corpuscles, while the latter are lined by

rows of flattened cells closely resembling an endothelium, Interspersed among the epithelium of the bronchi, branched connective-tissue cells were found, communicating by their processes with those of the mucosa, and thus forming a pseudo-stomatous tissue by means of which the lymph-canalicular system may be brought into communication with the surface of the bronchial mucous membrane. That such a communication does exist was proved by Sikorsky, who found that coloured particles introduced into the bronchi penetrated into the lymph-spaces of the mucosa.

Lastly, the perivascular lymphatics are described as originating in the walls of the alveoli by a lymph-canalicular system occupied by branched connective-tissue cells, whose processes often project between the epithelium lining the alveoli, and thus form pseudo-stomata, which permit of communication between the cavity of the alveoli and the lymph-canalicular system. The lymphatic trunks formed by the confluence of the capillaries which arise from the lymph-canaliculi accompany the branches of the pulmonary artery and vein, chiefly as distinct vessels running by their side, but often, especially around the smaller arterial branches, the lymphatic vessels are replaced by lymphatic lacunæ which communicate freely with each other. The arterial or venous branch was sometimes seen to pass directly through a lacuna, in which it thus becomes invaginated.

The pathological portion of the work begins with an account of the changes observed in the pleura pulmonum in inflammation. The endothelium was found to germinate around the stomata, more especially in chronic inflammation; and in the course of chronic pyæmia and artificial tuberculosis the changes found were, (*a*) thickening of the matrix of the pleura preceded by infiltration with lymphoid cells; (*b*) hypertrophy of the muscular coat in guinea-pigs, so that the meshes between the muscular bundles become much narrower, and even a continuous muscular membrane may be found in some parts; (*c*) the intermuscular lymphatic spaces and many subpleural lymphatic vessels become filled with lymphoid cells, derived, in all probability, partly from the germinating endothelium around the stomata, and partly from emigration from blood-vessels. These plugged lymphatics share in the formation of the characteristic nodules of artificial tuberculosis, so far as the superficial parts of the lung are concerned. The vessels at first only filled with lymphoid cells are converted into cords of adenoid tissue, by an outgrowth of their endothelial walls in the form of fine fibres, forming a reticulum between the cells. The cords leave

meshes corresponding to the superficial alveoli of the lung, which in early stages are filled with cells which are undoubtedly altered epithelium; and later, by cells indistinguishable from lymphoid cells. These nodules are to the naked eye at first rounded, grey, and transparent; later they increase in size, become of a more irregular shape, and their centre becomes opaque and caseous. This central softening extends gradually in all directions to the circumference, and the nodule which first forms a distinct prominence on the pleural surface, becomes depressed in the centre when softening is advanced.

In the substance of the lung Dr. Klein distinguishes granulations of three kinds. (1.) More or less well-defined nodules in connection with the walls of small bronchi. These he regards as simply hyperplasæ of the normal adenoid tissue of this part. They are found at a comparatively late stage and do not soften. (2.) Perivascular cords. These are developed earlier around the small arteries, at first as endolymphangeal follicles by plugging of lymphatics with lymphoid cells, and their subsequent conversion into cords of adenoid tissue, and then the cords become further thickened by a perilymphangeal growth. Changes also take place in the blood vessels themselves. The endothelium of the ultimate branches of the pulmonary artery is found to germinate so as very materially to diminish the lumen of the vessel. In larger branches the middle coat becomes laminated, and infiltrated by lymphoid cells which extend into the coats of the vessel from the perivascular cords; finally the capillary vessels in the alveolar walls become ultimately converted into nucleated threads. This change takes place only in the later stages of the process, subsequent to a thickening of the alveolar septa by encroachment of the perivascular cords. In the perivascular cords no caseous degeneration (softening) was ever found. (3.) The last kind of granulations is due to catarrhal pneumonia. The alveolar septa become thickened as above described, and the alveoli themselves become blocked at first by alveolar epithelial cells and their derivatives, giant-cells forming a prominent feature. The largest of these Dr. Klein believes to originate from a fusion of several epithelial cells, as their substance shows an indication of being divided into territories. The catarrhal changes finally spread from the alveoli to the infundibula and small bronchi. It is these catarrhal pneumonic granulations which undergo caseation, a process which spreads at last to the thickened alveolar septa.

The last chapter is devoted by Dr. Klein to an account of

acute miliary tuberculosis in man, based upon the examination of the lungs of seven children who died of this disease. He found that in early cases the tubercles were due to catarrhal pneumonia; the alveoli being found distended with a fibrinous material in which numerous lymphoid cells (emigrated colourless corpuscles) were imbedded, the structure of the alveolar wall was barely discernible, and its capillaries obliterated. In later stages the fibrinous exudation which occupied the alveoli gradually disappears by absorption, and becomes replaced by groups of cells which are mostly derived from the alveolar epithelium, or by one large multinuclear mass or giant-cell. The giant-cell is connected by processes with a retiform tissue infiltrated with lymphoid cells, which represents the alveolar septa. This is not true adenoid tissue, but is regarded by Dr. Klein, in agreement with Schüppel, as formed by cells derived from the giant-cells. The giant-cell finally degenerates into a mass of débris, sometimes passing through a previous fibrous stage. Dr. Klein considers that, in the lung, giant-cells are formed from the alveolar epithelium, though he admits that it is possible that they may arise (according to the observations of Ziegler) from emigrated colourless blood-corpuscles. In still later stages, when the above tubercles already show necrotic changes, numerous blood-vessels are found surrounded by perivascular cords, and spherical collections of adenoid tissue are met with in the adventitia of the bronchi, so that the various processes take place in man in inverted order as compared with the artificial tubercle of guinea-pigs.

The book is illustrated with six admirable double plates, and is certainly among the most valuable of Dr. Klein's numerous contributions to normal and pathological histology.

The Histology and Histochemistry of Man. By Heinrich Frey, Professor of Medicine in Zurich. Translated from the fourth German edition by Arthur E. J. Barker. London, 1874. (Pp. 683; 604 woodcuts.)

No working histologist who is acquainted with the German language needs any introduction to Professor Frey's manual. Avowedly a compilation, it is yet a very satisfactory and valuable compilation, and, especially for students' use, is perhaps the most useful text-book on the subject in any language. It is hardly necessary to say that it has gone through several editions, and as each successive issue has had to be brought up to the present day, as the phrase is, some parts present a curious patchwork of conflicting views, or

rather, perhaps we should say, of views arranged in successive strata, like a geological formation. Sometimes the successive views are very contradictory, and sometimes the contradiction hardly seems to have been perceived by the author; the latter portion having been tacked on without rewriting the original text. But this is probably unavoidable in a book which has been patched and altered so many times.

It is an unfortunate peculiarity of histology that so little appears to be positively established, and so much is a matter of "views." The fact appears to be that observers do not, after all, differ so much as to the phenomenon itself as in their interpretations of it. If they confined themselves to stating precisely what can be seen, there would be little or no controversy. But there is little chance of this happy unanimity ever being attained, for several reasons. In the first place, many phenomena are in themselves, strictly speaking, ambiguous, with our present means of observation. There is a certain limited number of possible explanations, each of which has certain points in its favour, but no one of which can be absolutely proved. Then, again, no one is content with a mere description of the phenomenon, because a mere appearance without any attempt at interpretation has no interest for us. We do not want to know about black shadows and dots, but about what the shadows and dots may be supposed to mean. So that histological controversy is not likely to come to an end just yet.

A remarkable illustration of the ambiguity of appearances is afforded by the changes of opinion with respect to so well-known a tissue as voluntary muscle. These are well illustrated in Professor Frey's work. He gives, first, a very clear statement of what were once two rival theories as to the ultimate composition of the muscular fibre (though the former of them can hardly be regarded as a rival now), viz. the theory that fibrillæ are the pre-existing essential elements of the fleshy mass; and Bowman's view, which regards it as made up of "sarcous elements." The latter conception has very naturally the preference, since it has certainly commanded the suffrages of most recent observers; and when compared with the fibrillar theory, it appears plain that only the authority of some popular teachers and writers of text-books could have given the latter so much acceptance as it had at one time, especially in Germany. Professor Frey gives a very good account of the further developments which the conception of sarcous elements has undergone in the hands of Krause, Hensen, and others, the history of which appears

to us rather instructive. In the first place, we have the cross line of the transparent space, or "the transverse plate of Krause," as it is now named. Now this tranverse plate was not only referred to by "the English observer Martyn and others," as Professor Frey says, but described by almost every English authority from an early date. A very distinct figure is given in old editions (but omitted in later editions) of 'Carpenter's Human Physiology' (we quote the fourth, of 1853) though certainly interpreted in a very different way; the clear space is seen divided by a tranverse line, and a similar light space figured at the *side* of each muscular element; appearances which led Dr. Carpenter to regard the muscular elements as cells. This view of the structure of muscle, Dr. Carpenter states in a foot-note, was published simultaneously by himself and by Professor Sharpey, both statements being founded on some preparations made by Mr. Lealand, the optician. The same fact had also been previously recognised by Dr. Goodfellow and Mr. Erasmus Wilson. So that the "transverse line" itself was anything but a novelty when attention was drawn to it by Krause. The latter observer, however, not only described, he founded chiefly upon that an elaborate theory of the structure of muscle, which, whether accepted or not, has always been regarded as possessing in a high degree the merit of originality. In Professor Frey's words:

"Krause holds a very peculiar view in respect to the structure of muscle. He regards the dark cross line just mentioned as the optical expression of a delicate transverse partition springing from the sarcolemma, which divides the interior of the muscle fibre into a number of discoid compartments built up one over the other. The contents of such a compartment would consist from below upwards of (1) half of a transparent transverse zone; (2) of a dark zone occupying the middle (*i. e.* of a transverse disc of sarcous elements); and (3) of another half of a transparent cross zone. Krause believes also in the existence of a delicate lateral membrane, investing closely the sides of the sarcous elements and ends of its transparent appendages, and uniting with the transverse membrane. In this way he supposes the elementary structures of the striped fibres to be formed—the so-called muscle caskets [or compartments]. In longitudinal rows they constitute the fibrillæ. This author also believes the clear longitudinal and transverse cementing medium to be liquid, and that during contraction the layers of fluid flow from the end surfaces to the sides."

Now, strange to say, even this peculiar theory of Krause

is not new. It has some resemblance to the old "cell" theory before referred to, but it appears to be nearly identical with that referred to by Dr. Carpenter in the following extract (*op. cit.*):

"A similar delineation had previously (before 1846) been published, however, by Dr. Goodfellow ('Physiological Journal,' No. iv), but his interpretation of the appearances was altogether different; for he considered the dark spaces as the 'sarcous elements' of Mr. Bowman, and regarded them as separately enclosed within partitions formed by internal prolongations of the external investing myolemma."

These are merely the "Muskelkästchen." Dr. Capentrer continues:

"By Mr. Erasmus Wilson, again, the appearances were described as leading to the belief that two kinds of cells exist in each fibrilla, a dark and a light; a *pair* of light cells, separated by the delicate transverse line just spoken of, being interposed between each pair of dark ones" ('Manual of Anatomy,' third edition, p. 162).

Substituting the word "disc" or "body" for "cell," this enumeration of the constituents of a muscle fibre agrees precisely with Krause's.

We do not quote these anticipations merely to show that "there is nothing new under the sun," still less to deprecate the labours of the eminent professor of Göttingen, but to illustrate the assertion made just now that many phenomena are truly ambiguous, and that the number of possible explanations is, with given means of observation, limited. One explanation gains currency in preference to another not only by emphasizing certain features of the object, but by suppressing or ignoring others. Nevertheless, the suppressed points are certain to come to light again, and when they are emphasized the dominant theory is overturned. Thus the see-saw goes on, one side of the question alternately predominating; though it is true each explanation, when it is revived, is never revived quite in its original shape: it is always more complete than in its previous state of existence. Finally, some new method or improved instrument of research makes it possible to explain the true and the false in each of the contending theories.

Whether this will be the case with regard to muscle, when the views of Engelmann and Schäfer come to be thoroughly worked out, and whether sarcous elements are to disappear as a merely provisional hypothesis, we cannot now discuss, nor can we speak of Henson's "middle disc" dividing the dark zone.

Another interesting point discussed in Frey's work is the structure of the gastric glands and their peculiar forms of cells—the "Hauptzellen," or "chief cells" of Heidenhain (*adelomorphous* cells of Rollett); and "Belegzellen," or "investing cells," *delomorphous*, of Rollett (strangely translated "*overlaying* cells" by Mr. Barker).

Heidenhain's observations on the differences seen in these cells in periods of rest or activity of the gland, are one of the most important connecting links between physiology and pure histology.

It is, of course, impossible to criticise a work of this kind in detail. We can only say that other parts seem to be as well brought up to the date of publication as those which we have mentioned.

In conclusion, we must cougratulate Mr. Barker on the accuracy and fluency of his translation, and on the faithful reproduction of the German woodcuts. As we have before complained of the costliness of the American translation of 'Frey on the Microscope,' we feel bound to state that this work, though very considerably larger and containing nearly twice as many woodcuts, is published at a lower price, and is at least equal, if not superior, in mechanical execution.

Outlines of Practical Histology. By WILLIAM RUTHERFORD, M.D., Professor of the Institute in the University of Edinburgh. London, 1875.

SINCE the first edition of this little work appeared in the form of notes in the number of this Journal for January, 1872, we are relieved from the necessity of subjecting them to any detailed criticism. It is right to say, however, that the original notes have been considerably expanded and improved, as well as made more useful by the introduction of figures. In its present state, Professor Rutherford's *brochure* forms an excellent guide for class work, and will be useful even for the solitary student who is bent on seeing things for himself. It has the great merit, for a scientific text-book, of being useless in the absence of the objects described: a quality which will render it quite unfit for the crammer or the crammed.

NOTES AND MEMORANDA.

Mr. Sankey, on a new Solution for Staining Sections of Hardened Animal Tissues.—Although the number of dyes recommended for use in histological investigation is already somewhat large, I cannot refrain from adding another to the list, which appears to present useful qualities in a combination not met with in any other dyeing material with which I am acquainted. I have already spoken in favour of this dye in a paper in the last number of the 'West Riding Reports;' but I had not at that time used it extensively for staining hardened sections, and was, therefore, not acquainted with the best method of using it. In the dry state the substance, under the name of aniline blue-black, can be obtained from the manufacturers, Messrs. Read, Holliday, and Sons, of Huddersfield.[1] It is a blackish powder, something like gunpowder in aspect. It can be dissolved in alcohol, but it is much more soluble in water. When dissolved in alcohol it will be found to make a very useful dye for hardened sections of animal tissues.

Owing to the great facility with which it dissolves in water, and the difficulty with which it dissolves in alcohol, the best way to get it in solution in the last-mentioned liquid is first to make a very concentrated aqueous solution, and then to pour it into strong alcohol. In this way it is very easy to get a solution strong enough to dye any texture in a few minutes.

I find the following quantities yield a solution of convenient strength:—·05 gramme of aniline black is to be dissolved in 1 or 2 cubic centimètres of water; when solution is complete 99 cubic centimètres of methylated alcohol are to be added; the mixture must then be filtered, and is ready for use.

[1] Or at 15, Fenchurch Street, London. The price of the dye is about 6s. per lb.

By using an alcoholic dyeing fluid one of the steps in the process of mounting an ordinary hardened section in Canada balsam is avoided, and as the transference of a thin section from alcohol to an aqueous dye is always more or less hazardous the advantage seems to be of some moment.

I am aware that an alcoholic solution of logwood has been prepared for dyeing sections; but this fluid requires that the section should be immersed in it for a space of four or five hours before a sufficiently dark colour is obtained; whereas the fluid I recommend does not take more than a few minutes to stain the section darkly.

With regard to its power of staining some structures in preference to others, I think that, speaking generally, it stands in this respect intermediate between logwood and carmine, for while it differentiates nuclei from their cells more than carmine does, it seems somewhat inferior to logwood in this respect.

It is, however, permanent, which logwood is not, and moreover its solution is not liable to decompose by keeping, and is not precipitated from its alcoholic solution by oil of cloves; hence it is less necessary to rinse the dye from the preparation before placing it in the essential oil. The dye, moreover, seems to have a special affinity for nervous cells, and on this account it will be found very useful in preparing sections of the spinal cord and brain.

The colour produced by this material is a blue grey.—HERBERT R. O. SANKEY, Undergraduate in Medicine of the London University.

Reinsch's Contributiones ad Algologiam et Fungologiam.—Those of the readers of this Journal who busy themselves with, or take an interest in, the broad range of Algology and Fungology will be possibly glad to have their attention directed to a descriptive work of comparatively recent appearance and of considerable scope, under the title of 'Contributiones ad Algologiam et Fungologiam,' by Professor Paul F. Reinsch, of Erlangen, in Franconia.

Of this work the first volume only has as yet been published; it is not a monograph, but descriptions with illustrations and explanatory details of new species of Melanophyceæ, Rhodophyceæ, and Chlorophyllophyceæ, as well as a few species of Fungi; the second forthcoming volume will be devoted to Phycochromophyceæ. Of Melanophyceæ 57 new species are described, distributed in 9 genera, of which 5 are new; of Rhodophyceæ 68 (with 4 new forms), distributed in 22 genera, of which 7 are new; of Chlorophyllophyceæ 51 new species (with 32 new forms), distributed in

24 genera, of which 4 are new; of fungi are described 15 new species, distributed in 13 genera, of which 8 are new.

Amongst the most interesting of these new forms of algæ are some belonging to each of the three great groups above mentioned, which are of a truly parasitic, that is, entophytic, habit, that is to say, they carry on their existence embedded, at least partially, within the tissues of larger forms. It is only a comparatively short time since algæ of this nature were recognised, and a *résumé* of the knowledge of them at the time was given in the 'Quart. Journal of Micr. Science,' n. s., vol. xiii, p. 365, 1873. To the small number already known Professor Reinsch has, in the present work, added considerably, and what would seem somewhat surprising is that their distribution would appear to be pretty wide, though the forms are, perhaps, difficult of detection.

Entonema (n. g., Reinsch), belonging to Melanophyceæ, embraces species inhabiting the tissues of both Rhodophyceæ and Melanophyceæ of larger and of lax structure, both superficially and in the internal parenchyme of the infected plant; sometimes occupying the intercellular spaces, sometimes penetrating into the interior of the cells. The parasite itself forms jointed filaments with fructification of Ectocarpeæ. Species referable to Ectocarpus itself are described as having rhizomatoid radicles penetrating into the living substratum. *Entonema intestinum* (Reinsch) produces zoosporangia, the zoospores standing in transverse series.

Actinema (n. g. Reinsch) is a new epiphytic Melanophyceous genus—its fructification unknown; the minute plant forms a disc of parenchymatously conjoined cells, to some extent comparable to Phyllactidium (Kütz.), or Coleochæte (Bréb.), *minus* bristles, flatly adhering to the surface of larger algæ.

In Plectoderma (n. g. Reinsch), referable to Rhodophyceæ, of which the fructification is also unknown, we have a structure morphologically quite comparable, and apparently of wide distribution.

Again, amongst Rhodophyceæ we have a new truly parasitic genus in Choreocolax (n. g., Reinsch,) described with several species. The frond is composed of two parts or portions—one an irregularly filamentous system, becoming expanded in the parenchyme of the infected plant; the other rising above its surface, and forming externally a convex or hemispherical, or nearly spherical, or irregularly-lobed cellular body; the cells of the immersed portion are more slender than those of the external outgrowth, or equal to them; those of the external part are variously figured, disposed more or less without order, or in subramose densely intricate

threads; sometimes the most external cells are the longest. Fructification not known.

These curious Rhodophyceans parasite, living half in and half out of the host-plant, occur on Delleserieæ, Polysiphonia, Sphærococcus, Gigartina, Rhodomela, Laurencia, Ceramium, the various examples coming from far-distant localities. The first observed representative of this genus was that named by the author *Choreocolax Polysyphoniæ*, found by him on *P. fastigiata*, taken on the northern coast of North America. Examples may possibly be found on our own coasts of these remarkable growths, if sought for by our marine algologists. Sometimes the mass of the parasite expands out considerably larger than the stem of the infected plant on which it grows, and causes a good deal of distortion of the constituent cells of the latter. These, for instance, in the form named *Choreocolax mirabilis*, growing in the stems and branches of *Rhodomela subfusca*, may become dislocated in some numbers, and in section can be seen more or less displaced and commingled with and surrounded by the cells of the parasite. The cells of the host embraced by the parasite are described as becoming much altered, their contents deprived of colour, and completely filled by a granulose matter, assuming, on application of sulphuric acid and tincture of iodine, a bluish-violet, the cuticular stratum becoming dissolved, but the primary cellulose membrane remaining unchanged; at the same time the contents of the cells of the parasite becoming deeply coloured a dusky purple, the primary membrane unchanged. The contents of the cells of the parasites appear, for the most part, as composed of a nitrogenous substance (albumen), those of the host of an amylaceous matter.

The author describes also a further allied genus—Syringocolax (n. g., Reinsch)—also strictly parasitic, and, as to habit, like the preceding, living partly within and partly without the host-plant. Here, as in Choreocolax, the plant is made up of two portions, one immersed in the parenchyma of the infected plant, and composed of very slender threads, passing in amongst the cells of the host-plant, the external portion (as in Choreocolax) rising above the surface of the infected plant, here by a short pedicel, is irregularly figured. The substance of this external portion is formed of heteromorphous threads, the lower densely intricate, subcontorted, very slender, the outer oval, disposed in thicker tubular threads, forming a regular cortical stratum, externally bounded by a rather thick continuous covering. Fructification seems to be Polysporangia produced by the cortical

layer of cells, and containing twelve to twenty broadly oval spores. Of this genus but one species is described, found on *Gelidium cartilaginum*.

Entocolax (n. g., Reinsch) contains but one described form; here the whole plant is entophytic, itself seemingly composed of slender cells radiantly disposed in series, and spreading in cavities of certain monstrous curiously-lobed or horned cellular excrescences of the host-plant (here *Bostrychia* growing on *Gelidium cartilagineum*), its fructification wholly unknown. This curious production, the author seems to think, may take origin within the cavity of a cell of the host-plant, but this is surely very questionable. At any rate the young and very minute cellular plant is wholly included by the adjacent parenchym of the infected plant. More advanced, the expanded cavity seems to be covered by a laminated lining, in which nidus is seated the parasite. The curious horned excrescences on the host-plant are similar to those sometimes induced by and accompanying certain other parasites appertaining to Choreocolax, and are more minutely cellular than the normal tissue. How propagation of this remarkable parasite (referable, like Choreocolax and Syringocolax, to Rhodophyceæ, but of uncertain position therein) can be effected remains a marvel.

Pseudoblaste is the name of a new genus (of three species) formed by Reinsch for certain obscure little productions growing on the surface of other Rhodophycean algæ, and forming little convexities composed of homomorphous cells, arranged in vertical or radiate series or irregularly scattered, and enclosed in a sharply bounded, nearly hemispherical or lobed, colourless matrix, closely adherent to the plant on which they live, but wholly without any organic cohesion therewith.

Coming to Chlorophyllophycæ, a new form, amongst some others, referred to Chroolepus, is found to be a true parasite living in certain Jungermannieæ. In many cases the author observed the parasite, not only in the intercellular spaces, but within the cavity of the cells. The cells become sometimes wholly embraced by the growth of the parasite and disjoined, such being mostly without chlorophyllaceous granules, and the plasma densely and finely granular.

The author establishes a new genus under the name of Chromopeltis, for certain forms living attached to the leaves of mosses; these are disciform, subcircular, or irregularly lobed, composed of irregularly-figured cells, sometimes running into radiant series; contents finely granular, and colour dusky green. The figures convey the idea of plants

not unlike young examples of such a form as *Coleochæte scutata* without bristles (Phyllactidum, Kütz.), as does possibly the *unnamed "nov. genus Ulvacearum"* described on p. 76.

Certain forms of Polyedrium, Sorastrum, Gongrosira, Chlorotylium, and Ulothrix are described, as well as Microthamnion, Œdogonium, and Bulbochæte.

An alga named *Spirogyra annularis* is described, which seems remarkable. In it the filament tapers towards the (upper) extremity. The cells of the cylindrical (lower) portion are very short, being, in fact, nearly one half broader than long, the cell-wall rather thick ; the endochrome forms a complete ring, slightly twisted. To judge from the figure, the endochrome of the upper or tapering cells is different, and forms a longitudinal axile band. Quere, is this truly a Spirogyra?

A puzzling little, very slender, filamentous form is described and figured, but not named, believed by the author to come under Cymatonema (by an error printed "Cymatopleura"), but having, he thinks, also possibly as much right to be placed to Zygnemeæ. The cells are elongate, comparatively rather thick-walled, more or less regularly hexagonal, colour bright green.

Actidesmum Hookeri (n. s., Reinsch) is a curious little alga, somewhat calling to mind *Sciadium arbuscula* (Al. Br.). The thallus is formed of families of a few spherical cells, each single cell at the summit of a short slender hyaline pedicel, several of which radiate in a whorl from the summits of a number of longer and thicker hyaline pedicels, which latter themselves radiate from a common centre ; propagation by zoogonidia. It thus seems to differ, so far as can be judged, from Sciadium by the aggregate family not standing on a substratum by a common pedicel, and by the ultimate cells being comparatively large and spherical, not elongate.

Cœlastrum (*Sphærastrum*) *verrucosum* (n. s., Reinsch) is a well-marked form, characterised by the external subacute verrucæ.

Characium Dyerii (n. s., Reinsch) has a singular habitat —on the backs of entomostraca—cells elliptico-oval, contents granular, amylaceous, colour pale green.

Of Desmidieæ several new and well-marked species are described, a few of which, however, are not named. *Cosmarium pseudomargaritiferum* (Reinsch) appears to be the same as *C. Portianum* (Archer). It has a very singular zygospore, globose, thick-walled, and covered by pits, that is, scrobiculate ; hence de Bary's figure ('Untersuchungen ueber die Familie der Conjugaten,' t. , f.) cannot refer to this species ; in fact, to that extent the zygospore of the

Cosmarium in question agrees with the zygospore of the otherwise very different *Xanthidium armatum*. *Cosmarium auriculatum* (Reinsch) seems to be, in part at all events, equal to *C. perforatum* (Lundell).

A new genus, under the name of Schizospora, is founded by the author for two forms, which, however, were previously described and named by Lundell—*Cylindrocystis diplospora* (Lundell) and *Penium didymocarpum* (Lundell). Viewed apart from the zygospore, and having regard only to the arrangement of contents of the parent-cells, these two forms seem generically distinct, but they have in common, no doubt, the double (or twin) zygospore, upon which circumstance the new genus is founded by Reinsch. But the far more common form, *Cylindrocystis Brébissonii*, it would appear, *sometimes* produces double zygospores (see 'Quart. Journ. Micr. Science,' n. s., vol. xiv, p. 423), though single zygospores are characteristic, and frequently enough met with in that species. Again, that well-marked species, *Closterium lineatum*, produces double zygospores, like those of the two species referred to Schizopora by Reinsch, very closely apposed, so much so as to become mostly mutually flattened by the pressure, but also, like them, readily separable when mature. Again, *Spirotænia condensata* has double zygospores; but during formation they stand somewhat apart, not closely apposed. The conjugation, in such cases, seems to be effected by the union of the half of the contents of one of the parent-cells with that of the corresponding half of the opposite cell; that is to say (supposing the parent-cells to stand in a vertical position), the contents of the two upper halves passing across to become mutually combined, the contents of the two lower halves simultaneously and mutually doing quite the same thing, If, as we think, this is really the process, the resulting spores are truly twin or double spores, not a single spore subsequently subdivided. Seeing, then, that at least four forms appertaining to the diverse and well-recognised genera, Closterium, Spirotænia, Cylindrocystis, and Penium, produce these double spores, it would seem as if the proposed genus Schizospora were untenable, or, at least, unnecessary.

The form recorded as *Euastrum gemmatum* (Bréb.) *forma*, appears a very well-marked one, and should seemingly rank as autonomous. *Onichonema læve* (Nordst.), 'Symb. Fl. Bras.,' found by that author in a collection from Brazil, is an interesting addition by Dr. Reinsch to the European list. This is an allusion to a few only of numerous very pretty forms described.

The author describes and figures a few abnormalities in some Desmidian species, consisting of the interposition of a monstrous growth between the semicells, without any subsequent constriction or division, and of misshapen lobules in the wrong place, mimicking those that occur normally in other situations. Such monstrosities now and again turn up.

In Fungi several new genera are established for curious forms occurring on and in the stems and leaves of mosses, ou algæ, &c.

The author adds not less than 131 plates, containing figures of all the new species described in the work. These, if not finely done, are graphic and expressive, and, together with over 100 quarto pages of letter-press, containing the various descriptions, form a valuable record of long and patient and successful labour; and the work will be no doubt welcome to cryptogamic botanists in this country.—Wm. Archer.

Change of Editorship.—Professor W. T. Thiselton Dyer has found it necessary to retire from the joint editorship of this Journal in consequence of his appointment as Assistant Director of the Royal Gardens, Kew. His place on the editorial staff is taken by Mr. William Archer, F.R.S., of Dublin, well known to the readers of this Journal.

Professor Cohn's Beitrage;—Bacterium rubescens.—We have received the third part of the contributions to botanical physiology, published under Professor Cohn's direction by Kern, at Breslau, 1875. At present we are not able to give an account of the contents, except to mention that it contains several important communications relating to the Bacteria, besides other papers on Volvox, Utricularia, and Aldrovanda by Professor Cohn. Professor Lankester wishes to state that he is not convinced by the evidence adduced by Cohn of the correctness of a separation of the forms included under *Bacterium rubescens* into the two species *Clathrocystis roseopersicina* and *Monas okeni*. The flagellum ascribed by Cohn to *Monas okeni* (the large Bacterioid plastids of *B. rubescens*) is parallelled by the flagella of *B. termo*, described by Dallinger and Drysdale. Professor Lankester had observed the *appearance* of a flagellum as described by Cohn in this form on treatment with iodine or magenta solution, but had not been (and is not now) able to satisfy himself that the apparent flagellum is really part of the plastid to which it is attached, since in the fluid, together with the red-coloured plastids, are always found very numerous filamentous forms (Bacillus and Vibrio) of great delicacy and length, which by acci-

dental contact with the red-coloured plastids *may* simulate flagella.

Mr. Worthington Smith's paper on the Resting Spores of Peronospora infestans.—By an unfortunate blunder on the part of the Woodbury-Type Company both the photographs on Plate XIX were reversed, and the upper one was also inverted in the mounting. The key (Plate XX), of course, fails in consequence to correspond with the photographs.

PROCEEDINGS OF SOCIETIES.

Dublin Microscopical Club.

July 15th, 1875.

Leaf-Structure in Sphagnum Austini, Sullivant, and *Sph. papillosum*, Lindberg.—Dr. Moore drew attention to the importance of minute microscopic characters in the discrimination of species, as exemplified in two related mosses, *Sphagnum Austini*, Sullivant, and *Sph. papillosum*, Lindberg, and he exhibited specimens of their leaves, pointing out the fringe-like marginal development of processes around the spiral cells of the former, as distinguished from the dot-like papillæ occupying a similar position in the latter. The former examples he had gathered at the Island of Lewes.

Differential characters found in the minute Structure of the Leaves in Pinus Nordmanniana, P. pectinata, and another probably new species.—Dr. McNab exhibited sections of the leaves of three Pines, viz. *Pinus* (Abies) *Nordmanniana*, *Pinus pectinata*, and a species from the Himalayas. The plant of the last is growing in the Botanic Garden, Glasnevin, and was raised from seeds received from the East India Company by Dr. Moore; it resembles *P. pectinata* in general appearance, but differs in the shape of the leaf and in the quantity of hypoderm present. *Pinus pectinata* differs from *P. Nordmanniana* in having the two resin-canals in the parenchyma of the leaf, and not (as in *P. Nordmanniana*) in contact with the inferior epidermis. The other form,—which, if it turn out to be a new species, might be called *Pinus Mooreana*—has the resin-canal in the parenchyma, as in *P. pectinata*, but has a much-interrupted hypoderm, instead of the almost continous layer met with in *P. pectinata*.

Marasmis Hudsoni exhibited.—Mr. G. Pim showed *Marasmis Hudsoni*, the long sharp bristles with which the pileus is beset causing it to form a very pretty low-power object.

Navicula divergens, form, exhibited.—Rev. E. O'Meara showed a form of Navicula similar to *N. divergens*, but differing in being regularly elliptical in outline. He had found this form occasionally in the Lough Mourne deposit, and recently in great abundance on a slide kindly supplied by Rev. George Davidson, of Logie-Coldstone, the material having been gathered in a lake in his neighbourhood, called Lough Canmore.

Passalurus ambiguus was exhibited by Mr. Booker.

A minute Rhizopod belonging to the genus Microgromia, Hertwig and Lesser, *probably distinct from M. socialis* (Archer), Hertwig and Lesser.—Mr. Archer desired to mention that, in connection with the plant he had formerly described (now as is proven erroneously) as a species of Dictyosphærium, Näg., under the name of *D. constrictum*, and of which he had shown the conjugated state at the meeting of the Club in May last, he had now discovered that the little pear-shaped colourless body he had so long noticed accompanying that alga and imbedded in its mucous matrix, was not, as he had once conjectured, in any way necessarily belonging to the alga, but was a veritable Rhizopod, and in fact, as it would seem, appertaining to the genus Microgromia, a genus founded by Hertwig and Lesser on his own *Gromia socialis*. This little organism, found along with the little alga referred to, had long puzzled him; it might or might not have some genetic connection with the alga—it might be anything, in fact. But *there* it was, a little pyriform, nearly "colourless" (but yet showing a very slight bluish tint) inert body, a frequent concomitant of the alga. In the very last example he had seen (strange to say, very shortly after Mr. Archer had taken the gathering yielding the conjugated specimens of the alga, it had abruptly disappeared from its site!) he had, however, noticed its possession of pseudopodia and a whitish nucleus (like that of *Microgromia socialis*), which latter took a high tint on the application of Beale's carmine fluid. Its linear dimensions are scarcely more than one third those of *Microg. socialis*, its pseudopodia very slender, branched, granuliferous, their movements *very* slow. If the alga with which this little Rhizopod *consorts* should again make its appearance, Mr. Archer hoped to obtain more examples and give it a closer study than he was able to do with so limited material.

Bacteria (Bacillus) *habitually forming a Nidus in Mucous Investments of Algæ*.—Mr. Archer directed attention to an alga of which he had wished for specimens at the May meeting in order to have contrasted it with the so-called Dictyosphærium, referred to above, but he then had no examples at command: this was *Cosmocladium Saxonicum*, de Bary, a plant, though widely distributed, very rare in its occurrence. This species, however, he had before shown to the Club, and he now merely brought it forward as a good example of a fact he had found pretty generally noticeable in algæ surrounded by a mucous envelope, but he likewise thought not generally (if at all) noticed. This was that imbedded in a vertical or radiant position in the matrix occurred numerous bacteria (Bacillus-form), which from their rather regular disposition imparted a radiantly striate appearance to the surrounding *halo* of mucus. Similar occur too in the mucous envelope of very many Desmidieæ as well as other algæ, and give that striate appearance often noticeable; in fact, the depth and density of this mucous investment seems to a certain extent specific in various Desmidieæ, and even the amount of *striation* due to the

presence of these Bacterian forms might be regarded as characteristic in certain species; so much so that a Cosmarium had been described by Mr. Lobb as "spinous," and possessing "rays," and named *Cosmarium radiatum* ('Quart. Journ. Micr. Sci.,' vol. xiv, p. 56), but Mr. Archer ventured to suspect erroneously so, and fancied the so-called "rays" might in reality be simply these striæ in the mucus. Sometimes these bacteria near the periphery become dislodged and "wriggle off" with themselves, but their movement is "lazy," comparatively speaking; sometimes a little pressure facilitates their removal and their becoming free. Similar bacteria also sometimes occur in the periphery of *Volvox globator*, but there they lie in a tangental position. Chlorophyllaceous algæ are seemingly more prone by a good deal than Phycochromaceous forms to thus harbour bacteria (themselves doubtless to be regarded as Phycochromaceous algæ), but the former, as a rule, have more mucous envelopes, and thus form a better nidus.

It must not for a moment be supposed the algæ are in any decaying or abnormal condition; on the contrary, the presence of the "bacteria" is most marked in the most healthy and vigorous examples, and in various forms (Desmidieæ and others) they are pretty constantly and more or less characteristically present. As to *how* these "parasitic" bacteria become developed in the position adverted to, Mr. Archer could not hazard a conjecture, but their comparatively regular radiant, though interrupted, linear disposition (around some globular forms, almost calling to mind under a moderate power some "Actinophryan") and their habitual presence seemed sufficiently remarkable; at any rate such a peculiar *nidus* for these indeed, in some form or other, omnipresent existences, Mr. Archer believed had hardly been noticed.

August 19*th*, 1875.

New Species of Synedra.—Rev. Eugene O'Meara brought forward a new species of Synedra, lately found by him in a swampy place on banks of Royal Canal, near Kilcock, Co. Kildare, and which he named *Synedra spathulata*:—Frustules very large, length 0·0130″, in front view wider at the ends than at the middle; greatest breadth 0·0012″, ends straight; in side view wider at the middle and gradually attenuated towards the ends, at some distance from which (0·0025″), bending inwards and then outwards, then suddenly constricted towards the broadly capitate rounded extremities.

Young of Water Snail exhibited.—Mr. Crowe showed specimens moving by their cilia still in ovo, a pretty example of "pond life."

Peculiar globular problematic enlargements of Mycelium of Penicillium glaucum hitherto unobserved.—Dr. McNab showed examples of the Common Blue Mould, *Penicillium glaucum*, which he had cultivated for fifteen days on moist bread. The mycelium had produced an abundance of conidia-spores, but in addition peculiar swellings of the mycelium had been observed. These

swellings are not noticed by Brefeld in his memoir on Penicillium and are therefore of interest. Sometimes they occurred singly, but in general there were necklace-like rows of them—six to eight together; the contents consisted of colourless protoplasm, with one or two nuclei. The swollen portions were generally surrounded by a thick mass of mycelium-threads, so that they became obscured; the swellings were globular, about $\frac{1}{3000}''$ in diameter, their size bringing them at once into marked contrast with the slender mycelium-threads which produce them. Dr. McNab did not hazard any conjecture as to their nature or function, but stated that they seemed somewhat to resemble the "Macrogonidia" of Penicillium figured by Hallier in his 'Phytopathologie,' Pl. v, fig. 31.

Resting-spores of Peronospora infestans exhibited.—On the part of Prof. Thiselton Dyer, who kindly sent the material, Mr. Archer exhibited some of the original diseased potato-specimens in which Mr. Worthington Smith had made his recent very interesting discovery of the resting-spores of *Peronospora infestans*. On looking over the slide, only one example of the "antheridium" was to be noticed in actual contact with an oogonium, but even in Mr. Smith's experience this was rare to find.

Fructification in Polysiphonia.—Dr. McNab showed a minute branch of *Polysiphonia fastigiata*, gathered at Seapoint in July, which showed the young cystocarps, and in two of them the minute lateral structure was developed, which he identified as the trichogyne. On the same portion of *Fucus nodosus* he had met with a *Polysiphonia* bearing antheridia on same plant and tetraspores on another.

Structure of Spines in Echinothrix Desorii.—Mr. Mackintosh exhibited transverse sections of the spine of *Echinothrix Desorii*, Peters. The spines of this species approach those of *E. calamaris* much more closely in structure than *E. turcarum*, both of which have been described in these Minutes. In *E. Desorii* there is a wide central cavity surrounded by a narrow zone of network through which pass the stems of the solid pieces, which are very elongated, with concave sides, and usually globular terminations. The stems are joined by broad transverse bars, and in some cases there are the remains of a network which extended over the central cavity. Mr. Mackintosh was indebted to Prof. Peters, of Berlin, for opportunities of examining the spine of this species.

Sections of Obsidian from Ascension Island were exhibited by Prof. Hull.

New Species of Euglypha.—Mr. Archer exhibited the test only, as the sarcodic portion had ceased to live, of what seemed doubtless a new species of Euglypha:—Test minute, but of variable size, ovoid, compressed; when old, brown, or reddish in colour (quite as highly tinted as *Arcella vulgaris*), sometimes seeming to incline a little to a purplish tint, when young, colourless; in the highly coloured examples paler near the opening; hexagonal facets *extremely minute*, elongate, test not prolonged into a "neck," its

opening bordered by indistinct teeth, but sometimes indefinitely terminated, even as if somewhat torn (with, as it were, an "unfinished" appearance there); no spines whatever; nucleus, with nucleolus, large, well-marked, posterior (as usual in the genus); a band of darkish granules across the middle portion. Its small size, the ovate compressed form of the test, without any neck, the reddish colour and very minute facets, seemed to render the form now shown a very distinct Euglypha. It occurs not rarely on Bray Head, but Mr. Archer had not noticed it from any other locality. In reference to its high tint, as compared to the other always colourless and hyaline species, Mr. Archer thought this form might stand as *Euglypha tincta*.

September 16*th*, 1875.

Sections of leaf of Pinus (*Tsuga*) *Sieboldii*, var. *nana*, exhibited.—Dr. McNab showed sections of the leaf of the foregoing species—the Finie—of the Japanese. It differs considerably from the typical form in having the young shoots hairy, a character not mentioned by Parlatore, the leaves much smaller and having well-developed hypoderm cells. The type-form has the shoots perfectly glabrous and developes no hypoderm except at the margins of the leaf. The characters observed seem to warrant the foregoing being considered a distinct species, bearing the same relation to *Sieboldii* that *Mertensiana* does to *Canadensis*, and *Hookeriana* to *Pattoniana*.

Peculiar globular problematical bodies occurring in diseased potato-leaves.—Mr. Greenwood Pim showed preparation of diseased potato-leaf infected with *Peronospora infestans* treated after the manner recommended by Mr. Worthington Smith, viz. maceration in water. There were to be seen a number of spherical bodies with somewhat rough or tuberculated exterior, the external coat of which showed a certain amount of tendency to split into three valves, and this circumstance, coupled with the fact that no direct connection of these with the hyphæ, which abundantly permeated the cell-substance of the leaf, could be detected, rendered the nature of these bodies problematical. They were at first taken for the resting-spores of the Peronospora, but that ceemed to be at best but doubtful. This indeed imparted to them probably even a more considerable interest. It was seemingly highly unlikely they could be any kind of pollen-grain, so thoroughly interspersed as they were in the substance of the leaf. Mr. Pim detected some much smaller colourless bodies, possibly the antheridia, alluded to by Smith, but could not satisfy himself on the point.

Nectria peziza exhibited.—Mr. Pim likewise showed a specimem of *Nectria peziza* in which the white spores were oozing in a globule from the orange spherical stromata, the whole forming a very pretty object for a low-power and reflected light.

Sections of Petioles of Nymphæa, species exhibited.—Mr. Mack-

intosh exhibited cross sections of the petioles of *Nymphæa alba* and *N. dentata*, and called attention to the differences of structure which obtained in the two. In *N. alba* the central part of the petiole is occupied by four moderately large lacunæ, which are nearly equal in size, one pair being somewhat larger than the other, and surrounding these are a series of smaller lacunæ; the whole of the passages are thickly studded with stellate cells. In *N. dentata* the central part is occupied by two very large lacunæ, with a pair of much smaller ones at each end of the septum which separates them; other still smaller lacunæ occupy the rest of the circumference; there are no stellate cells. Mr. Mackintosh hoped to be able to work out these interesting points more in detail, in order to determine if each species has a characteristic structure in its petiole. He also showed sections of the petiole of *Villarsia nymphæoides*, in which the lacunæ are provided with stellate cells somewhat like those of Nymphæa, but devoid of the peculiar tubercles which roughen the surface of the cells of Nymphæa.

Micrasterias angulosa, Hantzsch, *exhibited for the first time in Ireland.*—Mr. Archer showed *Micrasterias angulosa*, Hantzsch, for the first time, though he had long been acquainted with this species; the specimens were from Co. Westmeath. He showed also a specimen of this species from Professor Reinsch's collection, gathered in Germany, drawing attention to the complete identy of the examples.

Mr. Crowe showed, for comparison's sake, several species of Micrasterias taken lately in North Wales, such as *M. rotata*, *M. denticulata*, *M. papilifera*.—He also showed *Actinosphærium Eichhornii* occurring in the same gathering.

Cosmarium Reinschii, n. s., exhibited.—Mr. Archer further showed a Cosmarium which he had no doubt was identical with that recorded and figured by Prof. Reinsch in his 'Contributiones ad Algologiam et Fungologiam,' t. xviii, f. 4, but not named by that author. This Mr. Archer had also some time known, but it is quite rare; the present examples were from Co. Westmeath, and he was able to place side by side therewith, under another microscope, a specimen found on a slide in Reinsch's collection. These were perfectly identical, nor could he doubt that the form found in the Reinschian collection was that the author had in view (*loc. cit.*), but Mr. Archer thought his figure showed the lateral projections as too angular: they are rather undulately rounded. This species, which seems abundantly distinct, might well stand as *Cosmarium Reinschii*.

A Glimpse of a Vampyrella-form.—Mr. Archer spoke of what he would call but a *glimpse* of a Vampyrella-form and its doings; though time would not admit of his fully exhibiting some examples of the encysted condition merely (which he had put up temporarily with a little glycerine) of this so energetic little particle of sarcode, as well as so choice apparently in the selection of its "prey." The active "animal" was of a reddish hue, vacuolate (not unlike

Hertwig and Lesser's Leptophrys, 'Archiv für Mikrosk. Anatomie,' Band x, t. ii, f. iii), and its food the moving examples of a Chlamydoccus swimming about in pairs or fours. The little *vampire*—which was always altering its figure, and throwing out and withdrawing its slender, slightly branched pseudopodia—when it came in contact with one of these colonies, lay around one of the cells, embraced it, sunk into its outer coat, wrapped up the inner green cell with its own body-substance, then became itself encysted (in order to enjoy its *dinner*, unmolested, in comfortable and quiet seclusion, one would suppose!), and so remained for a number of hours (seemingly some twenty-four); by and by, the sarcode contents, "in the twinkling of an eye," suddenly burst the cyst and reappeared separated into four to five or six small Vampyrellæ, with the same characters of vacuolar, reddish (but paler), granulated sarcode, and the characteristic pseudopodia and movements. In the encysted condition, especially if indeed four Vampyrellæ (which was rare) had each "got hold of" the four component cells of a colony of the alga, the group would call to mind Cienkowski's description of his "Tetraplastic" forms, but this would apparently be a deception, for by far more frequently one only (sometimes two) of the component cells of the alga became the passive victim of this little vampire; such a partially attacked colony would still move about and carry its destructive visitor with it. When the wall of the encysted parasite lay empty the remains of the vegetable portion could be seen as a condensed black little body, as if so much of it was not capable of being "digested," and was thus left behind—as unassimilated fécal matter. Indeed within the encysted examples the alga substance could be seen in different degrees of alteration, from bright green to brown and black, and gradually becoming smaller in size. Mr. Archer could not dare to name this "rhizopod" or make sure of its individuality; it might just be a small representative of Cienkowski's own form. It was but scarce in the gathering and the supply soon ran out, and he regretted to say that all he could communicate was this brief record of his "glimpse."

Medical Miscroscopical Society.

October 15th, 1875.

Jabez Hogg, Esq., Vice-President, in the Chair.

The Cochlea in Birds.—Dr. Pritchard explained and exhibited specimens illustrative of the structure of the cochlea in birds.

Artificial Fibrillation of Hyaline Cartilage.—Mr. Cresswell Baber exhibited specimens illustrating his paper in the 'Journal of Anatomy and Physiology' on the above subject. His observations were based upon a statement by Tillmanns, in Max Schultze's 'Archives,' to the effect that fresh hyaline cartilage can be fibrillated by macerating it for several days in a solution of permanganate of potash, or in 10 per cent. solution of chloride of sodium. Mr. Baber showed that fibrillation of the matrix can be produced by macerating sections of hyaline cartilage in solution of chloride of sodium (both 10½ per cent.) in lime water or in baryta water, and in each case after the maceration applying momentary pressure to the glass covering the section before examining it. The fluid that acted most rapidly was baryta water, which produced the fibrillation in half an hour, while permanganate of potash, that Tilmanns prefers, he had found uncertain in its action. Mr. Baber had found the fibrillation of the cartilage matrix in all cases in which he had searched for it, and concluded therefore with Tillmanns that the hyaline matrix is composed of fine fibres held together by an interfibrillar cement substance that can be dissolved by certain coagents. A discussion followed, and the meeting then resolved itself into a conversazione.

November 19th, Friday.

Dr. Pritchard, Vice-President, in the Chair.

Micro-photography.—Dr. George Giles read a paper on, and exhibited an instrument for, quickly connecting an ordinary microscope with an ordinary camera, and obviating the use of the heleostal. The latter difficulty is met by using as a condenser an achromatic lens of large diameter, but long focus, say from ten to twelve inches. The image produced by such a lens is so large in comparison with the field of the microscope that one has simply to change the focussing screen for the dark slide, and to expose, before the earth has moved sufficiently to throw the light off the object. If the condenser has a diameter of three inches it will condense quite sufficient light for any power that one may want to use, say up to 1000 diameter. It consists of a firm base board, at one end of which is a stage to which a camera can be fixed by means of binding screws. In front of this stage is a sort of tramway, in which slide first a piece of wood, with

cavities to fit the feet of the microscope; next a stand to support the condenser and alum cell; finally a mirror moveable in all directions, the motion being performed by means of pulleys at the back of the instrument. The camera is connected with the microscope by means of a loosely-fitting metal tube, made proof against light by a lining of thick washleather. The instrument is used by placing it across a table in front of a window, so that the mirror projects out of it clear of all shadow. The condenser is focussed by sliding it along the tramway, the light thrown on by pulling with the string, and the exposure conducted as in all photographic manipulations, the time of exposure being as instantaneous as possible.

The process that answers best when the sun is tolerably constant is the ordinary "wet collodion;" but when it is being often obscured by clouds the "gelatin-bromide," in the form of "Kennet's patent sensitised gelatin-pellicle," a dry process, and by which micro-photographs can be obtained by the light of an ordinary paraffin lamp.

The Chairman suggested the use of a frosted silver mirror to supply a white light.

Dr. Matthews proposed using a paraffin lamp with double flame, and stated that, in order to save daylight, plates need not be developed at once, but could be put aside safely for some hours if melted with treacle and water. The yellow colour of the specimens, if it were requisite, might be corrected with hæmatoxylin.

Mr. Giles, in reply, preferred sunlight direct if it could be obtained, and did not find the colour of the slides any drawback.

Differential Warm Stage.—Mr. Golding-Bird explained and exhibited in action a simple form of hot stage, heated by a spirit lamp, capable of being kept in action for any length of time, its temperature being regulated according to the condition of pieces of solid paraffin placed on it, and on a copper tongue connected with it. He had found it extremely useful for purposes of demonstration, and its simplicity allowed of its being used in the wards of an hospital when examining blood in a morbid state.[1]

A discussion followed, and the meeting then resolved itself into a conversazione.

[1] This stage has been already described in 'Quarterly Microscopical Journal' for Oct., 1875, and is made by Millikin, of St. Thomas's Street, Southwark, S.E.

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MEMOIRS.

Observations *on the* Early Development *of the* Common Trout (*Salmo fario*). By Dr. E. Klein, F.R.S. (With Plate VI.)

In March, 1872, a paper was read before the Royal Microscopical Society, in which I described and illustrated the segmentation and spontaneous movement of the germ of the fertilized trout's ovum, as observed in the living condition. These observations, as far as the segmentation is concerned, were in accordance with the older assertions of Rusconi, Vogt,[1] Lereboullet,[2] and Coste, on Teleostean fishes, and were opposed to those of Stricker.[3] In that paper (which was printed in the 'Monthly Microscopical Journal,' May, 1872) I have stated that in the germ of the trout's ovum segmentation, at least in its earlier stages, is of the same regular type as in other Teleostean fishes, and that only in the more advanced stages the blastoderm presents the appearance as if the elements of cleavage—*i. e.* the embryo-cells—originated by being constricted off from the main body of the blastoderm, in a manner similar to that described by Stricker.

Independently and almost simultaneously,* Oellacher[10] described the movement and segmentation of the fertilized blastoderm of the trout's ovum, in a manner which is in complete accordance with the description given by myself.

In that paper I have also mentioned the spontaneous movements and the appearances of division presented by the embryo-cells, while in a living condition, between the fourth and tenth days. The same has been afterwards noticed also

* The Reporter on Embryology, in 'Jahresbericht über die Leistungen und Fortschritte in der Anatomie und Physiologie' for the year 1872, edited by Virchow and Hirsch, while making an imperfect abstract of an abstract that had appeared in this journal, leads the reader to assume—of course incorrectly—that my observations were made after those of Oellacher.

by Weil[9]* and Romiti.[13] The appearance of the segmentation cavity, the differentiation of the blastoderm, while growing round the yolk-sphere, into a thinner central and a thicker peripheral part, the foundation of the embryo, and the development of the embyonal layers, were described in accordance with Stricker and Rieneck.[5] The development of the central nervous system was mentioned in conformity with the assertions of Kupfer[4] on this subject.

Oellacher, in his paper[11] on the development of the embryo and the embryonal layers, has made some very minute and detailed observations, which to some extent tend to modify the previous accounts of these matters.

Thus, Oellacher shows that the segmentation or subgerminal cavity does not appear, at the outset, between the central part of the blastoderm and the yolk, but is founded excentrically, in consequence, as it were, of the peripheral thickening of the blastoderm being much greater and broader on one side than on the other. This statement I can fully confirm (see fig. 4). As this, however, does not form the chief subject of my present inquiry, I will not pursue it further.

Comparing thin sections of the blastoderm of different stages of early development which have been hardened in dilute chromic acid (one sixth per cent.), and then prepared in the ordinary way, I find that besides the blastoderm proper in its different aspects during early segmentation (compare figs. 1—4), the subgerminal and paragerminal substance deserves equally close attention, for this substance bears at all stages of development an important genetic relation to the blastoderm and the embryo.

As is well known, the blastoderm of the trout and of Teleostean fishes in general lies closely upon the surface of the yolk-sphere in a slight depression of the latter, round which most of the oil or fat globules accumulate immediately after fertilization. As segmentation proceeds, the blastoderm becomes gradually separated from the surface of the yolk-sphere by the appearance of a cleft, *i. e.* the subgerminal or segmentation cavity. From the earliest stages of segmentation—say, from a period when the blastoderm appears on section to be a finely granular mass, which, by the presence of a few more or less distinct perpendicular and horizontal fissures is divided into a certain limited number of contiguous

* The Reporter in the 'Jahresbericht,' while mentioning this observation of Weil, states, *anticipando*, that Romiti, whose researches were conducted in the Reporter's laboratory, arrived at similar results; while writing so the Reporter is altogether unaware of my own description of the same phenomena.

masses, of which those nearest to the yolk are largest, the fissures being distributed chiefly over the superficial part of the blastoderm—there is a uniformly granular substance to be noticed around the blastoderm, which, although connected with its (the blastoderm's) peripheral edge, forms no integral part of the blastoderm itself, and does not participate directly in the process of segmentation. It is this substance and its changes which I am going to describe in the present paper. Let us then commence with the examination of a thin section through the ovum of a trout of a very early stage of development.

[*Note.*—The ova which formed the material for the present research developed somewhat slowly, for only after nine days' incubation did the subgerminal cavity make its appearance.]

A vertical section through the blastoderm of the third day, which when viewed from the surface has the appearance of being divided into about twelve or sixteen elements, presents the following features :—The blastoderm is of the shape of a plano-convex disc, the free surface being plane or rathervery slightly depressed, the lower, *i. e.* that resting on the surface of the yolk, being convex ; the largest vertical diameter amounting to about 0·4 mm. The external margin of the blastoderm does not rest on the yolk, but is overhanging, as it were, like a lip, owing to the presence of a relatively deep groove (see fig. 1). A section comprising the largest horizontal diameter —which is about 1·15 mm.—would therefore cut the blastoderm in the external half of its largest vertical diameter, as is shown in fig. 1 ; the blastoderm is divided into a number of large elements, which are separated from each other by more or less distinct fissures. These latter, which in my specimens hardened with thin chromic acid, present the appearance of thin septa, are seen to penetrate only to a certain depth, the deepest mass of the blastoderm being free from them, *i. e.* being as yet undivided. This corresponds to what I have figured in my first paper (l. c., fig. 9), and what is termed by Oellacher[10] (l. c., p. 23) " basale masse." The blastoderm appears as a granular mass ; the granules, however, are not uniformly distributed through it, for in the depth they are much larger than in the superficial parts, and they gradually shade off into those contained in the superficial parts of the yolk. Nevertheless, the boundary by which the lower surface of the blastoderm is separated from the yolk of the saucer-like depression on which the blastoderm rests, is tolerably sharp (see fig. 1) ; the yolk of the saucer-like depression contains, besides large oil drops—occasionally,

but rarely, I have seen one or the other oil drop included also in the deepest part of the blastoderm—solid granules of a yellowish colour and of very various sizes (see fig. 1). Some of the last-mentioned elements appear in the specimens under consideration, as if they contained perfectly black specks (fig. 1), the number of which varies according to the size of the yolk-granules they are contained in; in the large yolk-granules the black specks are grouped together in the centre. In the blastoderm itself are found numerous yolk-granules containing these black specks; in fact, we are able to detect, with great ease, in the blastoderm the former by the presence of the latter, and in the same way we are able to state that towards the surface of the blastoderm the yolk-granules and their black specks decrease in size. Similar yolk-granules, containing these black specks, may be detected in the blastoderm also in later stages (see figs. 2, 3, 4, 5). Already Rieneck (l. c., p. 360) mentions the presence of yellowish yolk-granules in the cells of the blastoderm of the more superficial layers, and Oellacher[10] (l. c., p. 26) speaks of the elements of the blastoderm as if feeding (on the yolk-granules) "from mouth to mouth."

I have mentioned above that the extreme marginal portion of the germ does not rest on the surface of the yolk-sphere. By this I mean that portion only which is, so to speak, overhanging the paragerminal groove, for I have to add now that the substance of the germ extends below that groove outwards on the surface of the yolk. This extension of the germ is, from the circumstance above mentioned, only a continuation of the deeper part of the germ. It consists of the same granular mass, and includes also smaller or larger yolk-granules; it is thickest where it is attached to the germ, and becomes thinner in proportion as it becomes more distant from it (see fig. 1). This quasi-extraneous portion of the germ I will call *parablast*, in contradistinction to the segmented part or blastoderm of the authors, which I will term *archiblast*. These terms I do not use, however, in the sense in which they are applied by His ('Entwicklungs geschichte der Wirbelthiere; Entwicklung der Hühnchens,' Leipzig, 1868) to the ovum of hen, for, according to His, parablast is not a portion of the same substance of which the blastoderm consists, but is a part of the white yolk. Parablast and archiblast in the trout's ovum, however, is one continuous mass, *i. e.* one and the same substance, of which only one portion —blastoderm (Auct.) or archiblast, *i. e.* that lying in the saucer-like depression of the yolk—undergoes segmentation, *whereas the* second portion, parablast, not participating in

the process of segmentation, extends as a thin crust on the surface of the yolk outside the saucer-like depression, and, as will be shown afterwards, is important for the development of the layers of the embryo and some of its tissues. Oellacher,[10] who first correctly described (l. c., p. 12) that part which corresponds to our parablast—but who unfortunately calls it vitelline membrane, not knowing its great importance for the future development of the embryo and embryo-cells—mentions that it is a direct continuation of the germ, already before the germ undergoes segmentation. Although I have not been able to ascertain how far the parablast extends on the yolk-sphere, still I am inclined to accept Oellacher's very tenable proposition, that the ovum of trout is comparable to a fat cell, thus: the protoplasmic mantle of the fat cell consists of a thickened portion which includes the nucleus, and of the rest which is only a thin crust of protoplasm; the thickened nucleated part corresponds to the unsegmented archiblast or blastoderm (Auct.) with its germinal vesicle, while the rest of the mantle is represented by the parablast; to the oil drop of the fat cell included in that protoplasmic mantle, corresponds the yolk (food-yolk) of the ovum.

[Vogt[1] speaks of a vitelline membrane in the ovum of *Coregonus palœa*, which in the fertilized ovum is continuous with the blastoderm. He says, p. 29: "Le renflement (blastoderma Auct.) occupe invariablement le milieu du disque huileux . . . . et ses bords passent insensiblement à la membrane vitillaire qui à l'air de le recouvrir."

Lereboullet[2] (p. 127) says that an ovum which immediately after fecundation is coagulated by acidulated water contains an amorphous, granular, membranous pellicle which surrounds a third or half of the ovum, and includes also oil drops; it lines also the saucer-like depression and represents the future "feuillet organique" or "feuillet muqueux" of the embryonal germ, *i. e.* of the blastoderm, Auct., which lies above it, and which is very adherent to that pellicle. The "feuillet muqueux" does not participate in the segmentation (l. c., p. 128); it consists (l. c., p. 134) of a thickened periphery and a thin central lamella; while the blastoderm grows around the yolk it is accompanied by the "feuillet muqueux," which precedes, however, the former (l. c., p. 136). Later on the "feuillet muqueux" contains also cells, and enters into the formation of the wall of the intestine.

Kupfer[4] (p. 217) mentions the appearance of nucleated cells around the blastoderm on the surface of the yolk in the ovum of Gasterosteus and Spinachia at a somewhat late stage of segmentation. These cells are not derived from the

blastoderm, but develop, according to Kupfer, after the type of "free-cell formation," probably (p. 218) out of a fluid or semifluid blastema, comparable to that of insects (Musca, Chironomus); this blastema is situated in the periphery of the yolk, and in it appear at first nuclei—larger than those of the embryo-cells of the segmented blastoderm—around which the substance of that blastema gradually accumulates as "cell-substance."

Kupfer believes (p. 220) that this blastema is not only limited to that part where it was seen by him (Kupfer did not examine sections, but observed only fresh ova in surface and profile views), *i. e.* at the periphery around the blastoderm, but that it *extends over the whole surface of the ovum*, and is concealed by the blastoderm which gradually spreads (grows) over it. Thus Kupfer maintains that, independently of the blastoderm, a layer of cells appears which covers the yolk. Kupfer is unable to say whether this layer has anything to do with the formation of the hypoblast ("feuillet muqueux" of Lereboullet) or not.

Van Bambeke,[8] in examining vertical sections through the meridian of the ovum of osseous fish at the end of segmentation, finds (l. c., p. 1057): "la calotte blastodermique se compose de deux parties parfaitement distinctes, une supérieure, représentée par les cellules issues du fractionnement du disque, et qui entourent la cavité de segmentation . . . . . ; l'autre partie de la calotte blastodermique est formée par une couche . . . . qui ne prend point part au fractionnement." On account of this latter portion being situated *between* the segmented blastoderm and the yolk, Bambeke calls it "*couche intermédiaire*," and distinguishes in this "couche intermédiaire" a peripheral thick portion (bourrelet périphérique) from a central thin part (l. c., p. 1058); the former possesses in vertical section a triangular shape, and is therefore to be regarded as a prism bent into an annular mass and resting with one surface on the upper segment of the yolk-sphere; its external surface, *i. e.* that directed outwards, is free, and the upper surface supports the peripheral part of the segmented germ.

The central portion of the "couche intermédiaire" is a very thin lamella uniting the inner angle of the prismatic ring, and thus separating the segmented germ from the yolk.

Bambeke thinks that this latter, *i. e.* the central thin lamella, has probably grown from the periphery, as it is not present in earlier stages; it forms later on the hypoblast or "feuillet muqueux" of the blastoderma, and accompanies *this latter* while growing around the yolk.

Bambeke describes the "couche intermédiaire" as consisting of a granular protoplasm, the granules being much larger than in the embryo-cells. The thick portion of the "couche intermédiaire" includes constantly a certain number of nuclei and cells, which Bambeke believes to develop by endogenous cell-formation. Bambeke finally concludes that the "couche intermédiaire" is the lower portion of the germ-disc, split off from the latter in consequence of the fecundation; it does not undergo cleavage like the upper portion, *i. e.* the blastoderma (Auct.).*

From what has been said previously we can modify already now a portion of these statements. Another portion, that referring to the hypoblast, we shall have to discuss hereafter.

The only mention of cells outside the blastoderm made by Oellacher[1] refers to a "not inconsiderable numbers of cells" (l. c., p. 12), which having become separated from the lower surface of the blastoderm during the formation of the segmentation cavity remain on the floor of this latter (compare on this subject Rieneck's paper, my first communication on the development of the trout's ovum, and Stricker's article "On Development of Simple Tissues," in Stricker's 'Histology'), and "graben sich in die oberflächlichsten Schichten des Dotters ein." Besides this there are found cells also outside the region of the segmentation cavity; these Oellacher thinks may have migrated there from the former place, or may have become separated from the lower surface of the blastoderm where this is in contact with the yolk. These cells remain for a very long time in the yolk. Oellacher found them (l. c., p. 13) very numerous, even after the heart was formed and while the vessels of the yolk-sac are being developed; especially in the hind part of the embryo they are very numerous. They enlarge while the embryo proceeds in its development, and are of very various shapes. In longitudinal sections through the embryo these cells form long strips below the former; they also undergo multiplication. Oellacher further mentions (l. c., p. 17) that in certain stages of the development of the subgerminal cavity the most superficial layer of cells of the blastoderm extends for a short distance on the surface of the yolk beyond the blastoderm; this, Oellacher goes on

* This "couche intermédiaire" of Van Bambeke cannot be compared, as is done in the Reports in the 'Jahresbericht für Anatom. und Physiol.,' for 1872, p. 78, with the "basale masse" of Oellacher (see above), for this is only the deep part of the blastoderm, which undoubtedly undergoes segmentation though somewhat later than the part situated more superficially. The "couche intermédiaire" of Van Bambeke does not segment.

to say, may correspond to the layer of cells observed by Kupffer (see above) around the blastoderm of Gasterosteus and Spinachia. As we have mentioned before, Kupfer makes these cells originate after the mode of endogenous cell-formation.

Balfour,[14] in speaking of the later stages of segmentation of the ovum of Elasmobranch fishes, says (l. c., p. 4): "The limits of the blastoderm are not defined by the already completed segments, but outside these new segments continue to be formed around nuclei which appear in the yolk. At this stage there is, therefore, no line of demarcation between the germ and the yolk; but the yolk is being bored into, so to speak, by a continuous process of fresh segmentation." Balfour then goes on: "Intimately connected with the segmentation is the appearance and history of a number of nuclei which arise in the yolk surrounding the blastoderm. . . . . The blastoderm thus rests upon a mass of finely granular material, from which, however, it is sharply separated. At this time there appear in this finely granular material a number of nuclei of a rather peculiar character." These nuclei vary, according to Balfour, very much in size. "They are rather irregular in shape, with a tendency when small to be roundish, and are divided by a number of lines into distinct areas, in each of which a nucleolus is to be seen. The lines dividing them have a tendency to radiate from the centre." These very peculiar nuclei are scattered through the subgerminal granular matter; they are distributed in a special manner under the floor of the segmentation cavity, on which new cells are continually appearing, and are identical with those present in the cells of the blastoderm. From these facts Balfour concludes that the "*nuclei of the yolk*" become actually the nuclei of cells which enter the blastoderm; they are later on concerned partly in the formation of the vascular system, and still more in that of "the walls of the digestive canal and of other parts."

Haeckel,[15] in his studies of the development of Teleostean fishes (probably Lota), finds that only the cells resulting from the segmentation of the blastoderm participate in the formation of the developing body of the fish, all the rest of the ovum, *i. e.* food yolk, is homogeneous and amorphous, and has nothing to do with the development of the blastoderm. "Thus," Haeckel goes on to say (l. c., p. 96), "the false 'Parablast theory of His,' and all similar theories, according to which in *discoblastic vertebrate ova* histogenetic embryocells are said to develop out of the separate food yolk, *independently of the primary embryonal layers*, are hereby 'bündig

widerlegt.'" But only a few pages later (p. 102) he says, that there exist, however, other discoblastic ova in which that portion of the food yolk which lies next to the blastoderm participates also in the segmentation, and produces cells which become partly blood-cells, partly connective-tissue cells; in doing so Haeckel maintains that Goette "has *proved*" (Max Schultzse's 'Archiv,' vol. x, 1872) that in the discoblastic bird's ovum segmentation extends also into a portion of food yolk; in this are produced cells—"yolk-cells," which become used "partly as blood-cells, partly as food for the developing embryo;" likewise Haeckel takes it that Balfour "has shown" that, in Elasmobranch fish, a large portion of food yolk undergoes segmentation; and, finally, E. Ray Lankester ("Development of the Cephalopoda:" see this Journal, 1875, No. LVII) "has seen" in the discoblastic ova of Cephalopods numerous cells originate in the food yolk, which therefore undergoes secondary cleavage.]

We proceed now with our own observations.

The next stage I wish to describe is that represented in fig. 2. The archiblast consists of nucleated embryo-cells, the most superficial layer of which contains large cells of an opaque substance. The segmentation has proceeded (as is seen in fig. 2) to a considerable extent; the shape of the archiblast is that of a biconvex disc, thicker, however, at one edge than at the other; its thickness in the centre is over 0·5 mm., its breadth over 1·4 mm. The archiblast is in its whole extent resting on, but at the same time very sharply defined from, the yolk, which latter appears in hardened preparations as if limited by a membrane. Underneath the archiblast the yolk contains large oil drops and yellowish granules; the latter become larger in size, the farther away from the surface of the yolk. The paragerminal groove above mentioned is still distinctly seen. Next to this groove, and in immediate contact with the archiblast (see fig. 2) extends the parablast outwards on the surface of the yolk, gradually becoming thinner as it becomes more distant from the archiblast. The most conspicuous part is the thickened portion of it (parablast), which, as is shown in fig. 2, presents in vertical section a more or less triangular shape; that is, it corresponds to a prismatic body, of which Van Bambeke (see above) correctly observes that it surrounds the archiblast like a ring-shaped rim. But Van Bambeke is not correct in saying that this mass has an outer, inner, and lower surface, for it has an outer, inner, and upper surface; nor that it extends as a thin layer over the saucer-like depression of the yolk; and

likewise he is not correct in making it stop at an outer angle, for this prismatic annular mass is only the thickened portion of the original parablast. For the sake of simplicity, however, we will henceforth consider under the name of parablast only this prismatic rim. Its greatest thickness is about 0·12 mm.; its lower surfaces, *i. e.* its internal and external surface (contrary to the assertion of Bambeke) rest on the yolk, with which they are in very close connection, the parablast being, as it were, moulded into the jagged surface of the yolk; the upper surface of the parablast is free, and appears in hardened sections bordered by a very sharp outline as if by a membrane.

The substance of the parablast is a finely granular material, containing isolated, minute yolk granules, which become larger nearer to the yolk; on account of this the line of boundary between parablast and yolk cannot be at all points made out with precision. In the very superficial parts, and also at some places of the deepest parts, I think I can recognise faint outlines of what corresponds to nucleus-like bodies.

In a later stage (fig. 3) the archiblast is seen to be still biconvex (slightly thicker at one edge), and much broader than in the former case (over 1·5 mm.); its thickest diameter being, however, smaller (0·4 mm.). The most superficial layer of its cells is very conspicuous, and appears almost separated from the rest by a thin cleft. This separation, as we shall see, remains persistent in the following stages, and to it is due the differentiation of the corneous layer from the nervous layer of the epiblast (see Rieneck's paper, my first communication, and Stricker's article, mentioned above).

The archiblast is very well defined from the yolk of the saucer-like depression. The yolk possesses the same morphological characters as in the former case. The parablast, however, has changed considerably: (*a*) it has become more finely granular and transparent, thinner, and at the same time broader; it resembles now a mass of a more placoid shape, which is wedged in for a certain distance (see fig. 3) between the archiblast and yolk; at the same time bearing the same close relation to the yolk as in the former case. That this wedge-like process of the parablast is due to an active ingrowth of the latter, and not merely to the circumstance that the archiblast, while growing in breadth, spreads *over* and *beyond* the inner edge of the parablast, is clearly proved by comparative measurements; (*b*) in many sections *its superficial* layers are, over greater or smaller areas, of a

more or less distinctly fibrillar structure especially well shown in the part next to the archiblast; (*c*) it contains a great number of well-defined vesicular nuclei, many of which contain a nucleolus; they (nuclei) are most numerous in the parts near the edge of the archiblast, and are of different sizes and shapes—chiefly elongated in the superficial parts—and appear generally to be disposed in groups, forming chains of bodies more or less pressed against each other. To these nuclei we shall return more minutely hereafter.

[*Note.*—The fibrillar structure just referred to is of such a character that the apparent "fibrils" run in a direction parallel to the free surface. Balfour mentions the presence, in osmic acid specimens, of a fine network of fibrils "in any part of the fine granular yolk around the blastoderm," that is, in the portion comprising "the nuclei of the yolk."]

Next in order is a blastoderm twelve days old, which in vertical section exhibits a distinct subgerminal or segmentation cavity. This is seen to be situated excentrically in conformity with the assertion of Oellacher. The archiblast is thicker in the periphery than in the centre. The former is not of uniform thickness, for at one side it is much thicker than at the other (see fig. 4); this latter corresponds to that part of the periphery towards which the segmentation cavity extends. The central thinner part of the archiblast is several cells deep; they are more or less polyhedral, and contain one or two nuclei. At the periphery there exist underneath these, in addition, layers of cells, which are more opaque, containing numerous yolk granules; they are of different sizes, and possess not unusually a nucleus in the state of division or two or more nuclei; also to the lower surface of the thin part of the blastoderm is attached one or the other cell of this character.

The parablast is seen to have increased considerably in extent (see fig. 4). Although under a low power only the thicker portions of it are discernible, yet the examination with a higher power proves that it not only underlies the peripheral part of the archiblast, but that it extends as a thin lamella on the surface of the yolk for a considerable distance towards the zone of the segmentation cavity. Thus, in the preparation which is represented in the present figure 4, I can discern, under a moderately high power (Hartnack, fig. 7), a thin layer of parablast extending on the right side beyond the peripheral thickening of the archiblast for some distance underneath the segmentation cavity; whereas on the left side a thin layer of parablast may be followed on the surface of the yolk, close to the

edge of the segmentation cavity. The peripheral thickening of the archiblast rests on the surface of the thickened parablast, both being in very close contact. Sometimes (see the left side of fig. 4) the boundary between the two is indistinct, and then it appears as if the deepest elements of this part of the archiblast were directly developed out of the parablast, especially if one bears in mind that numerous nuclei are contained in this portion of the parablast, which (nuclei), as will be more minutely described hereafter, are similar to those of the cells of the archiblast.

Whether this definite boundary between the two structures (parablast and thickened part of archiblast) is a sufficient reason for saying that the deepest cells of the archiblast *of this stage* develop out of the parablast—the substance of the latter becoming differentiated round its nuclei into cell-territories—is a matter which I am not in a position to decide definitely; it is, however, very probably so, inasmuch as in only a little later stage cells may be followed with certainty to develop out of the parablast. This will be stated hereafter.

From the inspection of fig. 4 it is also clear that the thickened part of the parablast is not situated symmetrically in all parts in relation to the archiblast, *e. g.* on the right side it is situated more externally than on the left. This thickened portion of the parablast contains very numerous nuclei; they are more numerous in the superficial than in the deeper parts.

In a somewhat later stage (thirteen days) the archiblast is seen to be separated, for nearly its whole extent, from the yolk by the segmentation cleft (see fig. 5). The elements of which the archiblast now consists are little smaller than those in the former stage, except the most superficial layer, which is composed of slightly flattened cells: all the other layers are made up of polyhedral cells. At and next to the peripheral thickening of the archiblast, there are present on its lower surface numerous more or less spherical elements loosely connected with each other; some of them are much larger than the ordinary embryo-cells, and possess two, three, and more nuclei, besides yolk granules. I have seen some of these cells contain as many as six nuclei, each with a nucleolus. These nuclei were arranged like the sections of one large lobed nucleus. But also in other parts, on the lower surface of the archiblast, isolated cells may be found of the same character as those just mentioned (see figs. 5, 6, 7, and 8). Except the difference of the most superficial layer of cells, the archiblast does not present as yet any differentiation of its elements into strata.

The parablast is seen to have assumed much greater

dimensions. First of all it is found to possess a considerable thickness, not only around the peripheral thickening of the archiblast, but also at the peripheral part of the floor of the segmentation cavity; and, secondly, with a high power the parablast may be traced to extend for a considerable distance towards the centre on the surface of the yolk as a thin granular lamella, including nuclei, and possessing here and there swellings, which appear to be wedged in between the large oil globules of this part of the yolk. There is little to be added at present with regard to the nuclei of the parablast, except that in the part surrounding the edge of the archiblast they (nuclei) are elongated and form extensive rows.

A section taken at a right angle through the peripheral part of the specimen represented in fig. 5 naturally shows the segmentation cavity included between the archiblast and the thickened parablast, the lower surface of the former showing the loosely connected elements of various sizes, and the latter being conspicuous by its uniformly granular substance and the larger or smaller groups of its nuclei.

It may be mentioned already here, that amongst the elements lying loosely on the floor of the segmentation cleft (see above), there are seen here and there apparently free nuclei, some of considerable dimensions, and resembling nuclei contained in the parablast.

At a somewhat later stage (fourteen days) the thin part of the archiblast consists only of two layers of cells, very distinctly separated from each other (see fig. 6), the upper layer (corneous layer) being continuous with a similar layer at the thickened periphery of the archiblast, and the lower with what corresponds to the nervous or second layer (see my first communication on this subject). In the peripheral thickening (of course, including that portion of it which becomes transformed into the embryo) there are present underneath the two strata just mentioned several layers of cells, which, as has been stated already, form a less continuous mass than the superficial cells. The parablast is found to have increased considerably, not only in thickness, but also in breadth, as it may now be traced very distinctly over almost the whole surface of the yolk, *i. e.* almost along the whole extent of the floor of the segmentation cleft. As in the preceding stage so also in this, the greatest thickness of the parablast is no more to be found externally to or directly underneath the edge of the archiblast, but internally to it, *i. e.* on the peripheral portion of the floor of the segmentation cleft. Towards the centre the parablast becomes thinner, and in some places it is just possible to trace it from one

cuneiform accumulation to the other, wedged in between the fat globules of the yolk.

As regards the later stages, I may now be very brief. In all stages I found the parablast present as more or less continuous membranous masses, forming at numerous places cuneiform or prismatic or irregular shaped accumulations, not only underneath the embryonal portion, but also in all other parts of the archiblast. Its (parablast) aspect remains always the same, *i. e.* a finely granular material containing isolated, or, as is more commonly the case, groups of nuclei.

We have thus far been able to ascertain that one part of the germ, viz. the parablast at first situated outside the area of the archiblast (blastoderma, Auct.), gradually encroaches on the surface of the subblastodermic yolk, due partly to the growth of the blastoderm over the parablast, and partly due to an active growth of this latter itself, so that after a certain time the subblastodermic or segmentation cleft becomes lined on its floor with parablast.

There remain now two other points to be discussed: (A), the substance of which the parablast is composed and the nuclear elements found in it; and (B) the relation of the parablast to the cells on the floor of the segmentation cavity and to the elements of the archiblast in general.

(A) The substance of the parablast is, as has been mentioned on several previous occasions, a finely and uniformly granular substance, showing this slight difference in the earlier and later stages, that it is more transparent in the latter than in the former. (In this, as in the preceding, we mean by "parablast" only that portion which is at first situated in the vicinity of the edge of the archiblast, and, as has been shown, gradually grows inwards underneath the archiblast, so as to line the floor of the segmentation cavity.)

Whether the appearance of fibrillation which is to be observed in some parts of the surface of the parablast is due to a real fibrillar arrangement of its substance, or is only produced by the hardening reagent, must remain undecided.

The outlines of the parablast are not well defined towards the yolk, especially when the parablast has grown inwards on the yolk of the saucer-like depression; this is chiefly due to the fact that the yellowish granules which are contained in the yolk—yolk granules—are gradually shading off into the smaller granules contained in the parablast. To suppose that on account of the deepest part of the parablast containing granules which insensibly pass into the larger granules of the yolk, the two substances, *i. e.* parablast and

yolk, pass into each other, would be no more or less than to admit that the "granules" form the decisive character of the structures in question, and that the "substance of the matrix" plays only a secondary part. The parablast we are dealing with is a living organism; it grows from an inconsiderably small body into a structure of very great dimensions—dimensions which relatively are not very inferior to those of the archiblast; and that therefore this living structure (parablast), which is almost semifluid and has no limiting membrane, should, while growing on the surface of the likewise soft yolk, which has no limiting membrane either, take up the granules contained in the latter and thus feed itself, is just what we should expect. Under these circumstances it is clear that the line of boundary between the two structures must be lost, *i. e.* obscured by the granules; for the granules contained in what belongs to yolk pass directly into the granules contained in what belongs to parablast. The fact that the parablast has, at the outset, been forming one unit with what represents the archiblast (blastoderm, Auct.), and *while increasing, has spread*, i. e. *grown over the yolk* which underlies the segmentation cavity, is, I think, the most absolute proof that the yolk is as much different from the parablast as it is from the archiblast.

The nuclei contained in the parablast are different as regards shape and size. In the sections of hardened specimens which we are dealing with, only very few appear quite circular; many are roundish, with their sides more or less flattened on account of being pressed together in small groups; others are oblong, especially those about the periphery of the archiblast; and others again are either hour-glass shaped or kidney shaped, or *lobed*, furrows and notches extending for various depths into their substance; that these nuclei, therefore, multiply by division into two or more smaller nuclei is, no doubt, to be inferred from the frequent occurrence of chains of nuclei and of groups of nuclei of different sizes, in which the individual elements appear as sections of one large lobed nucleus. The largest nuclei which deviated least from the circular form were measured in a preparation represented in fig. 6; they were found to be 0·012 mm. in diameter. From nuclei of this size down to the smallest bodies which by their outline and connection with a distinct nucleus could be just recognised as such, all intermediate sizes could be detected. The substance of these nuclei is different from that of the parablast itself: being more transparent, the granules contained in the nuclei irregular and of very different sizes; besides this, each nucleus possesses a sharp outline as if

bordered by a definite, though delicate, membrane. There are, however, present in the parablast also true cells, *i. e.* granular corpuscles (more transparent than the parablast itself), containing two, three, or more bodies, which, either all or only some, possess a complete similarity with the nuclei of the neighbouring parts of the parablast. That masses of substance of the parablast separate themselves from the rest and collect round the nuclei so as to form "nucleated cells" is, I think, the simplest way of explaining the facts just stated. The question at present of greatest importance is, how do the first nuclei of the parablast develop? The circumstance that nuclei are not perceptible in the parablast in the earlier stages of segmentation, as mentioned on a former page, although not absolutely proving, renders it at least very probable that they originate *in situ*, as development in general proceeds. Besides, there is one definite fact I have been able to ascertain in somewhat later periods (*e. g.* in preparations like the one represented in fig. 5), which is apt to throw some light on the origin of these nuclei. It is this: searching carefully through the substance of the parablast with a moderately high power (Hartnack's No. 8), we detect numerous *isolated*, small, transparent bodies *very faintly outlined*, so as to be rendered just perceptible; between these and *distinct* nuclei *all intermediate forms may be met with as regards general aspect, outline, and size. This obviously means new formation of nuclei.* It therefore stands to reason to assume that, inasmuch as at a period when nuclei may be seen to multiply by division the formation of nuclei *de novo*, as it were, still takes place in the parablast, *the first nuclei of the parablast have also originated in the same manner*, i. e. *de novo*.

From the fact that some of the nuclei of the parablast possess elongated or knob-like processes of different sizes, which in some instances are more or less constricted off from the main mass of the nucleus, it may be said that the multiplication of nuclei takes place also by gemmation or budding.

The appearances of multiplication described by Balfour of the "nuclei of the yolk" in elasmobranch fishes (l. c., p. 4; compare also fig. 1 of his paper), as quoted on a former page, are not identical with those of the parablast of our case; whereas the appearances described and figured by Oellacher[10] (figs. 30—36) of the nuclei of the archiblast during early segmentation more closely correspond with those of the nuclei of our parablast.

(8) On a previous occasion we mentioned that on account of the intimate connection, at certain points, between the

peripheral thickening of the archiblast and the parablast, it appears probable that the deepest cells of the peripheral thickening of the archiblast owe their origin to that part of the parablast. In examining preparations like those represented in figs. 5, 6, 7, and 8, it may be easily ascertained that many of the cells lying on the floor of the segmentation cavity, especially many of those forming a loosely connected deep stratum of the peripheral thickening of the archiblast, are formed out of the parablast. Looking, for instance, at a preparation represented in figs. 7 and 8, it requires no difficulty, I think, to find not only nuclei perfectly identical with those of the parablast, but also larger masses, which in all characters denote their origin from the parablast. In fig. 8, especially, these facts come out with certainty; that huge element represented in the figure as attached to the lower layer of cells of the archiblast cannot conceal its place of birth; and the same may be found on close inspection with many large multinuclear elements, not only on the floor of the segmentation cavity, but also on the lower surface of the archiblast. It thus follows that cells of the deeper layer of the archiblast—in the peripheral thickening (including, of course, the embryonal shield), as well as in the central thinner parts—are derived, directly or indirectly, from the parablast. The assertions, therefore, of Rienek,[5] myself,[7] and Stricker (Stricker's 'Histology,' see the chapter on development of simple tissues), that the peripheral thickening of the archiblast owes its origin, to a great extent, to a displacement of elements at first situated on the floor of the segmentation cavity, but originally derived from the archiblast; and likewise the assertion of Oellacher,[11] that all the elements of the peripheral thickening owe their origin to the continuous reproduction of its cells, *i. e.* those contained in the peripheral thickening; and, finally, the assertion of Götte[13] and Haeckel,[15] to the effect that the deeper layers of the archiblast owe their origin entirely to an ingrowth of the edge of the archiblast (Götte, l. c., p. 684: "Der Rand des Keimes schlägt sich auf einer Seite um und breitet sich an der unteren Fläche des Kenines aus"); all these assertions are but partially correct. I cannot express myself with anything like certainty on this supposed ingrowth of the edge of the archiblast as maintained by Götte, and to some extent accepted also by Balfour; but this much I have seen, that the appearances presented by the peripheral thickening in general, and the embryonal shield especial, do not strictly necessitate the assumption of an ingrowth of the archiblast. On the other hand it is certain, as we have shown, that the deeper layers of cells of the archiblast are in

a considerable measure genetically derivatives from the parablast. This last fact is also insisted upon by Balfour, who, however, incorrectly, as we have proved, considers the parablast as a part of the yolk.

Now, the deeper layers of the peripheral thickening of the archiblast are, as is well known, in the embryo—which is developed out of a portion of that peripheral thickening—used for the formation of the hypoblast; from this and from what I have shown with reference to the parablast, the assertions of Lereboullet (feuillet muqueux), Kupfer, and Van Bambeke, with reference to the alimentary canal, receive their due significance.

From the presence of parablast, after a certain stage, beneath the whole extent of the archiblast—embryo and yolk-sac—it is only natural to draw the inference that within the area of the latter, *i. e.* the yolk-sac, notorious for the development of blood and blood-vessels, the parablast is concerned in the formation of blood and blood-vessels. But whether the elements derived from the parablast and contained in the deeper layers of the embryo are destined to be also material for the formation of connective and kindred tissues, is a conjecture which requires further consideration.*

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* P.S.—After this paper was in print I received the full monograph of Van Bambeke's research on Teleostean fishes. His observations on the earlier stages of development have not been noticed in this paper, since they are not contained in his earlier communication.

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Recent Researches *on the* Nuclei *of* Animal *and* Vegetable Cells, *and especially of* Ova.

By John Priestley, Assistant Lecturer on Physiology, The Owens College, Manchester. (With Plates XI & XII.)

While much important knowledge of the protoplasmic bodies of cells had rewarded the labours of histological workers, only a few ascertained facts, together with various ill-supported theories, existed respecting the intimate nature and the functions of the nuclei. During the present decade however, much of the energy of research has been directed to repair this deficiency; and the body of fact which we possess concerning nuclei is rapidly gaining in size and solidity. Some of the publications which have assisted in the impulse are those of which an account is now to be attempted.

Notwithstanding some marked diversities of theory, the general agreement in the statements of the various authors seemed to suggest that it would be of advantage to the English reader, and certainly fairer to the authors themselves, to give firstly a separate, brief *résumé* of the work of each, and afterwards to collate the various statements and indicate their points of divergence.

The historical notices, and all the references, are given on the responsibility of the several writers, since it has been impossible from many causes to check them. They are for the most part taken from the admirable critical article of Hertwig.

Among the first of the works referred to is that of Professor Auerbach, of Breslau, published in 1873-4.[1] In it there is recorded a very extensive series of observations

[1] 'Organologische Studien.' Breslau.

upon nuclei, their nature, microchemical reactions, and growth.

Nuclei in a perfectly recent and normal state, are described as flexible and elastic vesicles with a somewhat thick, highly refractile, doubly contoured membrane which appears to be less distinctly separated from the cell protoplasm than from the interior of the nucleus. This membrane is regarded as having been formed about the original drop-like nucleus by the differentiation of the inmost layer of protoplasm into a species of interior cell membrane. The main body of the nucleus is bright, and is called by Auerbach the *ground substance*. It includes one or more nucleoli, smooth bodies of irregular outline, as well as numbers of extremely fine *intermediary granules*. These are either impartially scattered throughout the nucleus, or arranged so as to leave a clear zone about the nucleoli, and sometimes also a similar zone beneath the nuclear membrane (Plate XI, fig. 1, a—d).

When nuclei liberated from the cell-body were submitted to the action of certain reagents, characteristic changes of appearance occurred. Water, added gradually, by irrigation, to nuclei so prepared causes, as a first effect, a shrinking, associated with the expression of droplets of a hyaline body which is supposed to exist in the interstices of the nuclear ground substance. These droplets cling to the irregular circumference of the nucleus and fill the hollows due to shrinking (Plate XI, fig. 1, e—g, m). The intermediary granules swell up and most of them become invisible; the interior of the nucleus assumes a darker, shining, almost homogeneous appearance, the distinction of wall and contents becoming less marked; while the nucleoli at this stage are very slightly affected.

If irrigation with water be continued the nucleolus begins to swell up, filling the whole of the nucleus and fusing with the membranous wall (Plate XI, fig. 1, l). If several nucleoli coexist in the same nucleus they may present different degrees of resistance to the action of the water. In the mean time the nucleus has attained to its original size; but continued exposure soon results in increased dimensions. When this point has been reached, the nucleus successfully resists any further destructive action of the water, not, as is generally thought, becoming completely disintegrated. Some of the later steps in the above process may be retraced by treating the nucleus with a 1 per cent. solution of NaCl, when nucleoli and the nuclear wall become again visible, showing that no true solution has occurred.

The first contraction of the nucleus is called by Auerbach *water-shrinking*,[1] while the subsequent tumescence of nucleoli is styled an *internal swelling* of the nucleus.

Solutions of salts and of acetic acid act upon nuclei in a manner which varies according to their percentage strength. For example, so-called indifferent solutions of Na Cl (·5—1 per cent.) produce a greater distinctness of nucleoli, intermediary granules and nuclear wall, which is in reality a *hardening* of the nucleus. Solutions which are more dilute produce a *shrinking* like that above described; below this, again, shrinking gives place to *internal swelling* as the characteristic alteration (Plate XI, fig. 2 B); while solutions containing very little salt indeed produce the final result of the action of water, viz. the *general swelling of the nucleus*. Above the point of indifference the action of solutions resembles that of solutions below it, the stronger solutions producing an internal swelling of the nucleus like the weaker solutions; while the strongest solutions have an action like that of the indifferent strengths themselves (Plate XI, fig. 1, h, i, k). These facts suggested a method of classification of the various strengths of solution which is illustrated in the following table:[2]

	NaCl.	$K_2Cr_2O_7$.	Ā.		
Upper region of hardening	35 p. c.	10 p. c.	60 p. c.		
	14	2	1·2		
Upper region of internal swelling	14	2			
	1·5	1·5			
Lower region of hardening	1·5	1·5	1·2		
	0·08	1·2	0·05		
Lower region of internal swelling	0·08	1·2	0·05	Shrinking of nucleus.	Swelling of nucleoli.
	0·008	0·03	0·01		
	0·001	0·006	0·001		
Region of general swelling	0·001	0·006	0·001		
	0·000	0·000	0·000		

Sugar in all solutions, from the weakest to those which contain 60 per cent., causes the nuclei to swell up; and

[1] Cf. Heat-rigor.

[2] In this table the figures represent the percentage strengths of the solutions used. The whole range of strengths is divided into regions, each characterised by a mode of action upon nuclei. Thus, of solutions of common salt, all those whose percentage strength ranges from 35 per cent. to 14 per cent. exert that action on nuclei which is termed *hardening*.

it may be used to counteract the shrinking effect of dilute solutions of acetic acid.

The above statements, although made primarily concerning the nuclei of carp's liver, may be understood as applying to nuclei in general, whether still within their cells or not.

With respect to nucleoli Auerbach has many new facts. Instead of accepting 1—5 as the limits of numbers in which nucleoli may occur, he regards 0—16 as being more correct; but he adds that even 100 may be seen in some nuclei. The enucleolar condition, besides occurring as a sign of degeneration in the shrunken and thickened nuclei of horny and some connective tissues, is seen in the early developmental stages of various ova (of mammalia, articulata, and vermes), as has been known since the time of Bergmann.[1] Auerbach has shown that this condition obtains in the batrachian ovum until cleavage has reduced the surface to a finely granular mass. After a few days, however, certain nuclei may be seen to have an ill-defined central cloudiness, which, growing darker and darker, becomes at last the nucleolus. In some cases the central haziness seems to radiate towards the periphery: hence it is supposed that the nucleolus is formed by the aggregation about the centre of nucleolar substance which is derived either from the periphery of the nucleus itself or from the inmost layer of cell protoplasm.

Besides increasing in the above manner, nucleoli may multiply by fission. Of this there can be little doubt, for Auerbach noticed a constant inverse relationship between the size and the number of nucleoli within the nuclei. If two nucleoli were present they were constantly smaller than the nucleolus in uni-nucleolar cases; while if three, five, &c., were to be seen, some were distinctly larger than the rest, as if the unevenness of number were due to the fission of some only of the nucleoli. This division is associated with a movement of the new nucleoli through the nuclear ground-substance, the cause of which is not understood (Pl. XI, fig. 2 A).

In addition to an increase in the number of nucleoli, the converse operation was observed in certain cases, viz. the fusion of several nucleoli into one.[2]

The functional significance of the increase of nucleoli is not apparent. In the epithelium of the stomach and intestine of the frog, *Rana temporaria*, the multinucleolar condition is constant only during the height of summer and in the au-

[1] Müller's 'Archiv,' 1841.

[2] This occurs in the fat-bodies and salivary glands of *M. remiforia*.

tumn, giving place to a paucinucleolar state during spring and winter. Again, it cannot be merely a phenomenon of the fission of nuclei, since it is seen in nuclei which are not about to divide (*e.g.* of red blood-corpuscles), or in those which are about to disappear (*e.g.* of the upper layer of epidermic cells). In the larva of *Musca vomitoria* the layers of cells which, in the pupa stage, undergo histolysis are just those which are characterised by an extraordinary growth of nucleoli. Hence it is, perhaps, not impossible that in many cases the multinucleolar condition may be a vain effort of nature in obedience to tendencies which have long since ceased to be of use, and the aims of which are only here and there fully realised, as in the case of *Musca vomitoria*.

We are now in a position to understand Auerbach's views respecting the origin of nuclei. According to him they first appear as clear spaces—vacuoles filled with a tenacious fluid mass—possessing no distinct wall. Each droplet then acquires a membrane by differentiation of the inmost layer of the cell-protoplasm, and nucleoli and intermediary granules afterwards make their appearance. Once differentiated the nuclear membrane is an integral part of the nucleus, constituting the latter a true vesicle, isolable as a whole by mechanical means.

Many facts speak for the identity of nucleolar substance and cell-protoplasm. In optical appearance nucleoli and the substance of young cells agree together. Vacuolation may occur in either, large nucleoli being seldom free from clear spaces. Both are amœboid, the movements in nucleoli having been described by many observers.[1] Lastly, nucleoli have, as Auerbach himself shows, that characteristic of vital protoplasm, the power of multiplication by division. In other words, nucleoli have all the capabilities of elementary organisms, and are in truth *cytodes*. Regarded in this light, they are real daughter-cells which have arisen by an endogenous process; and the nucleus is the chamber in which they develope. It is now merely necessary for them to find a way out through the body of the mother-cell in order to begin life as independent beings.

That this method of increase has become obsolete in the cases of a majority of cells of higher adult animals, or perhaps only occurs in pathological processes, may be quite true without offering any obstacle in the way of such a hypothesis. In the specialisation of function which appears as we ascend

[1] Metschnikoff (Virchow, 'Archiv,' Bd. xli). Balbiani (Keferstein, Jahresber. f. 1865, in 'Zeitsch. f. rat. Med.,' xxvii. La Valette St. George (Max Schultze's 'Arch.,' Bd. ii).

the animal series new means of gaining the same end present themselves, while the old vanish or are made use of for other purposes. The phases in the act of propagation of a unicellular organism may be less significant and have a far different outcome in a unit-cell of a more complex creature.

In examining the origin and growth of nuclei, Auerbach chose two objects which had already been studied by various previous observers, viz. the ova of *Strongylus auricularis* and *Ascaris nigrovenosa*, two species of parasitic nematodes. The protoplasm of these elliptical or elongated ova contains yolk-granules; and in the specimens which have already acquired a yolk-membrane, a clear fluid—*liquor ovi*—appears to have been expressed into the space immediately subjacent to it, collecting in larger quantities at the extremities. For the purposes of examination the ova were subjected to pressure caused by the capillary adhesion of the cover-slip and glass slide, a pressure which can be easily regulated by varying the size of the cover-slip and the quantity of fluid beneath it. By this means the obstruction to clear vision due to the granules was to a large extent removed; while the clear peripheral layer of liquor ovi became less or entirely disappeared. The specimens were removed from the body of the nematode and mounted in iodized serum.

If observations are commenced a short time after fertilisation the ova are seen to consist merely of protoplasm and of yolk-granules, the germinal vesicle having entirely disappeared. Soon the yolk-granules seem to have withdrawn from the periphery, leaving the latter quite clear. Thereupon the differentiation of the future yolk-membrane begins, either as a true secretory process, or perhaps, merely as a hardening of the outer layer of protoplasm. The completion of the yolk-membrane is a signal for the redistribution of the yolk-granules in the clear zone,—an act, which is, however, immediately followed by a contraction of the whole protoplasmic mass and the expression of the before-mentioned liquor ovi. This terminates the formation of the primary cleavage mass (Pl. XI, fig. 3).

At this point commences what Auerbach aptly calls the prelude to the cleavage-drama. At two opposite points of the periphery of such a primary cleavage mass—generally at the poles, and immediately below the yolk-membrane, clear, star-like, ill-defined spaces are seen to develope (Plate XI, fig. 4). These quickly grow larger and rounder, attaining a diameter of 12—15 μ, and their borders become sharper. Meanwhile certain pale, round, sharply defined nucleolar structures appear in their interior, giving them the

characters of nuclei which are not as yet provided with a wall. From their places of origin these peripheral *pronuclei* (to use Van Beneden's expression) move towards the centre with slowly increasing velocity, leaving behind them a clear track as of protoplasm destitute of granules (Plate XI, fig. 5). Duirng this migration, which is probably rather the result of contraction or movements of the surrounding protoplasm than of forces driving the bodies through a resistant mass, the nucleoli exhibit a marked activity, changing their relative positions in various directions and with varying velocities. At the centre the pronuclei touch, at first at a point, but afterwards, owing to mutual compression, along a straight line which runs in a direction across the long axis of the egg. The mass thus formed then rotates about its centre, so as to bring the previously transverse line of contact longitudinal (Plate XI, fig. 6), while the nucleoli one by one dissolve and vanish. The double structure begins to elongate in the direction of the greater axis (Plate XI, fig. 7), in the course of which elongation the dividing line of contact suddenly disappears along its whole length—a phenomenon which affords a strong proof of the absence of any definite pronuclear membrane. This completes the coalescence of the pronuclei, and the resulting rhomb is the true nucleus of the primary cleavage mass.

Cleavage proper now begins. Elongation of the nucleus continues in its former direction and a spindle is produced. Soon the tips of the nucleus exhibit clearer spaces, which radiate out in all directions into the granular protoplasm and are united one with the other by two clear lines, one on each side of the main body of the nucleus (Plate XI, fig. 8). In the mean time the latter has become smaller and more fissure-like, and it finally disappears altogether, leaving the twin-stars with an intermediate band (Plate XI, fig. 9).

The granules of the proper cell substance now begin to recede from a portion of the wall and a lateral inversion of the margin occurs (Plate XI, fig. 9). This rapidly runs into a furrow which extends around the circumference of the ovum—an operation in which the yolk-membrane is not involved. The furrow deepens inwards and finally constricts the protoplasmic mass into two.

While the furrow is still shallow two vacuoles appear in the band uniting the stars, one on each side of, and close to, the plane of the equator, and commence a movement towards the centres of their respective halves. Meanwhile the stellate figures in each half shrink, and, as they do so, the newly formed vacuoles increase in size. On reaching their re-

spective destinations the nuclei, which are about 15-18 μ in diameter, acquire nucleoli ; but as yet they remain quite naked.

Division into two may now be said to be complete. What remains of the bistellate figure gradually disappears, the nuclei alone remaining ; and activity is succeeded by repose.

The following stages of cleavage are but a repetition of the above-described steps. Division into four, into eight, and into sixteen masses can be traced in detail with comparative ease ; but beyond this the size of the objects renders observation difficult.

The theory by which Auerbach explains these appearances is one of a distribution and recollection of nuclear substance. After the disappearance of the original germinal vesicle and the occurrence of fertilisation, two nuclei appear, formed, as, in Auerbach's view, all nuclei are formed, viz. by a vacuolation of the cell mass. These slowly move together, touch, fuse into one, and pass into an enucleolar state. The resulting body, whose method of formation did not fail to suggest to Auerbach the resemblance to conjugation, elongates and becomes smaller, expelling its contents from both ends into the surrounding granular protoplasm ; and the disturbance in the arrangement of the granules gives rise to the appearance of two stars. This act is what Auerbach describes as *karyolytic* (*καρυον* = nucleus.)

The formation of the new nuclei is but a reversal of the above operation. The scattered nuclear substance in each half of the ovum aggregates about a point situated in what will shortly become its proper segment. It is, in reality, a return of the nuclear mass to its former state of aggregation after a temporary sojourn in the interstices of the cell ; and Auerbach has therefore designated the process of growth of the young nuclei *palingenetic*.

Another work of importance bearing out the life-history of nuclei is one by Prof. Strasburger, of Jena,[1] published in 1875.

In it three methods of the increase of cells in the animal and vegetable kingdoms are investigated, viz. free cell-formation, cell-division, and the renewal or rejuvenescence of cells (*Vollzellbildung*).

Of the first method several observations are recorded and figured, of which that of the embryo-sac of *Phaseolus multiflorus* is typical. There, in the formation of endosperm-cells, the nuclei first appear as punctiform thickenings, surrounded by a clear zone with tolerably defined borders.

[1] 'Ueber Zellbildung und Zelltheilung.' Jena.

(Plate XII, fig. 1). Both zone and nucleus increase in mass, but the zones in the denser portions are smaller in relation to the nuclei than in those which are less dense. The protoplasm of the sphere enclosing the nucleus often presents an undoubted radial arrangement (Plate XII, fig. 4). After a time the nucleus becomes vacuolated, the hollows being filled with fluid of a rosy tinge. The outer contour of the sphere soon differentiates into a skin (*Hautschicht*), while the remaining protoplasm of the sphere becomes reticular as the cell grows (Plate XII, fig. 5). Not until neighbouring cells have increased so as to allow of the direct contact of their skins is it possible to demonstrate the presence of a separating cellulose wall. After this stage has been reached and the cells form a complete tissue, all further increase takes place by cell-division.

Of cell-division in the vegetable kingdom the case of certain cells in the embryonal vesicle of *Picea vulgaris* may be taken in illustration (Plate XII, figs. 7—12). On fertilisation, the original nucleus of the embryonal vesicle disappears and four nuclei simultaneously present themselves at the upper extremity (the lower in the diagrams) of the vesicle. These are separated from one another and from the rest of the vesicle by series or rows of granules (Plate XII, fig. 7).

Hereupon a process of true division begins. The border of each nucleus becomes circular and ill-defined; and in the equatorial plane a disc or plate appears composed of a single layer of upright, parallel, rod-like granules. On either side of this *nuclear disc* (*Kernplatte*) the substance of the nucleus is marked by striæ which converge towards the poles of their respective halves (Plate XII, fig. 9), and terminate there in a somewhat circular spot.

The nuclear disc commences to thicken by the longitudinal extension of its rods, the latter being drawn out into thin fibres at their centres, while their ends travel in a direction along the converging striæ above mentioned, to meet together at the circular polar spots. Here they form clear ellipsoid structures of small size, which are in reality the nuclei of the future segments (Plate XII, fig. 9).

They do not long remain clear, but soon exhibit a striation of granules in the direction of their shorter axis (Plate XII, fig. 11). They surround themselves with zones of protoplasm and increase in size.

Meanwhile the *nuclear fibres* (*Kernfäden*) present central swellings which afterwards fuse into a second equatorial disc or plate (Plate XII, fig. 10). This plate grows in area,

thus causing a greater divergence of the nuclear fibres connected with it, and then splits into two laminæ—the skins (*Hautschichten*) of the coming cells, between which cellulose is developed. Hence this plate is called the *cell-plate* (*Zellplatte*). Division is completed beyond the circumference of the cell-plate by a like differentiation of the protoplasm existing there.

The above-mentioned phenomena of division, which constitute a favorable illustration for comparison with division in animal cells, were only studied in alcoholic preparation in the case of *Picea*. No doubt of their continuity can, however, exist, since in the large-celled freshwater alga, *Spirogyra orthospira*, Næg., with its suspended nucleus and cavernous or tunnelled cell-masses, the similar phenomena may be followed with great ease in the living plant under the microscope.

In the animal kingdom, where division seems to be the only well-authenticated mode of increase of cells, Strasburger examined the ova of *Phallusia mamillata*.

On the disappearance of the original nucleus a peripheral lenticular clear body developes. Of this the central part dips into the substance of the ovum and divides off as the new nucleus, which soon presents a vacuole containing nucleoli. The nucleus of the first cleavage mass, therefore, is not here formed by the union of two or more pronuclei (Pl. XII, figs. 13 and 14).

With the appearance of the clear disc the cell-protoplasm had become radiate, the rays proceeding from the disc; now, the radiation centres about the nucleus.

The latter soon becomes homogeneous and elongates; and finally exhibits a striation of a few converging lines with equatorial enlargements (Pl. XII, fig. 15). Strasburger did not himself succeed in observing an actual splitting of a nuclear disc; but Bütschli, in the cases of *Cucullanus elegans* and *Blatta germanica*, has figured this stage unmistakeably (Pl. XII, figs. 17 and 18)[1]. The new nuclei are formed much as in plants.

The stellation of the cell-mass is now twofold, proceeding from two centres; and certain of the rays from both centres intercross about the middle of the ovum. The protoplasm gradually clears up in the equatorial plane, whereupon division commences as an inversion of the edge of the ovum at the periphery of that plane, and is often completed as a sudden act.

The rejuvenescence of cells takes up but a few pages of

[1] Stasburger had Bütschli's permission to insert a few figures from an *unpublished* work by the latter.

Strasburger's work, and consists mainly of observations confirmatory of previous knowledge.

The similarity in detail of the process of cell-division in animals and in plants which his researches so fully display were to Strasburger suggestive of a community of descent of animal and vegetable cells. This suggestion cannot be regarded as being weakened by the existence in plants of other methods of cell-development; for, as Strasburger repeatedly insists, on the ground of the occurrence of various intermediate conditions, as well as from the functional analogy of similar parts, free cell-formation is not an original mode of increase, but has probably been derived from the method of division by the suppression of certain stages.

As to the nature of the nucleus itself, Strasburger differs entirely from Auerbach, in that he regards that organ as a solid structure closely allied to the skin (*Hautschicht*). The diversity of forms exhibited by nuclei, and the complexity of their functional processes, seem to the former observer quite to exclude an hypothesis according to which they are merely fluid-filled vacuoles.

In his views of the forces concerned in cell-formation Strasburger is at one with those[1] who consider them as exerting an attraction on the surrounding protoplasm, by means of which the latter is collected about the nucleus. Mere physical surface-tension, such as determines the shape of a portion of liberated protoplasm existing in a fluid medium with which it is hardly miscible, is, therefore, not concerned in the formation of cells.

A third recent publication, concerned chiefly with the phenomena of cleavage, is by Dr. Oscar Hertwig.[2] The cells examined were the ova of the sea-urchin, *Toxopneustes lividus*, fertilisation being effected by artificially mixing in a watchglass the male and female generative elements.

The granulous unripe ovum is enclosed in a porous capsule, and contains a germinal vesicle with definite walls, which in its turn includes a germinal spot of grey, compact substance capable of staining deeply in carmine. In addition the germinal vesicle possesses an interior reticular arrangement of protoplasmic threads.

About the time of fertilisation the capsule becomes converted into a doubly contoured membrane which has a clear substance immediately subjacent to it. The germinal

[1] Pringsheim, Sachs, Fol.

[2] Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des thierischen Eies. Morpholog. Jahrbuch 1.

vesicle undergoes a regressive metamorphosis, appearing first at the periphery of the ovum as a lenticular body of punctate mass, and embedding, in addition to the germinal spot, certain larger irregular structures, which do not stain readily in carmine.

After a time the germinal spot is no longer visible in the degenerate vesicle; but, instead, there is seen in the cell-body a clear round structure with no membrane, but with the capability of deeply tinging when treated with carmine. This body, which Hertwig calls the nucleus of the ovum (*Eikern*), never appears while the germinal spot is yet within the vesicle, from which circumstance, in addition to its deportment towards staining fluids, it is supposed to be the escaped germinal spot. Finally, the vesicle becomes entirely lost to view.

After fertilisation has taken place a small clear spot appears at the periphery of the ovum below the yolk-membrane, about which as a centre the yolk-granules commence to arrange themselves in radiate lines. Within the light space a small dark spot is seen having a like reaction to carmine with the nucleus of the ovum. From subsequent phenomena, and from the fact that he occasionally saw a delicate streak extending from the clear spot towards the periphery of the ovum, Hertwig believes the spot to be the head or nucleus of the fertilising spermatozoid, and accordingly he has named it the nucleus of the sperm (*Spermakern*). The two nuclei (the pronuclei of other observers) approach and meet near the centre of the ovum, where, after the nucleus of the ovum has exhibited certain variations of outline, due possibly to amœboid movement, they fuse together, while the dark central spot of the sperm-nucleus disappears from view. The resulting structure is called the nucleus of the first cleavage mass or first cleavage nucleus. About it a clear substance gradually collects, giving its outlines a faintness or indecision, while itself elongates into a spindle. The collecting clear substance aggregates chiefly about the tips of the spindle, towards which also the radiate strie of the cell-body converge in two sets.

There is, in fact, seen the bistellate figure characterised by Auerbach as karyolytic. The stars increase in size, the rays extending to the circumference of the ovum; and the nucleus between them, suddenly becoming indistinct, seems finally to disappear.

Division of the cell-substance now follows in the manner already described, being preceded by amœboid movement, a circumstance which Auerbach has also mentioned. The *young nuclei* seem to originate as described by the latter

observer, viz. as vacuoles in the intermediate band of the bistellate figure; and it may be ascertained that the total mass of the nuclei after division is larger than before.

Nothing more could be made out by examination of fresh ova. After hardening in $\frac{1}{10}$ p. c. solution of perosmic acid for two to five minutes and staining in carmine a further differentiation may be seen. The elongating nucleus is marked by longitudinal rods with thickened centres, which together form a *median thickened zone*. The nucleus, moreover, continues long after the time of its seeming disappearance in the fresh condition, extending as a band from the centre of one star to that of the other, and exhibiting at this stage *two lateral thickened zones*, one midway between the centre and each end. The clear portion of the band situated between them gets longer as cleavage proceeds, and the lateral thickened zones at last become the young nuclei.

As the result of his observations, Hertwig concludes that the nucleus is an automatic centre, situated within the cell-body and equipped with active forces. These forces determine the changes of form which occur immediately after fertilisation, viz. the amœboid movement and the elongation of the nucleus as well as the formation of the various thickened zones, which must be regargded as due to an aggregation of nuclear substance (*Kernsubstanz* as distinct from nuclear fluid, *Kernsaft*) similar to that observed in the growth of nucleoli. By means of other internal forces the nuclei are capable of assimilating to themselves material derived from the general cell-mass—a conclusion rendered necessary by the constant increase in the mass of nuclear matter after division. Other forces, again, acting on the surrounding protoplasm, cause a density in the neighbourhood of the nucleus which occasions the radiate arrangement of granules so characteristic of cell-division.

With regard to the final act in the latter process, viz. the segmentation of the cell-mass, Hertwig considers the forces at work to be proper to the protoplasm and quite independent of those acting on the nucleus.

In addition to the above complete researches we have before us a preliminary communication by Van Beneden[1] published in the year 1875.

As no extended research in mammalian embryology had appeared since the time of Bischoff, it seemed to Beneden that

[1] 'La Maturation de l'œuf, la Fécondation et les Premières Phases du Développement Embryonnaire des Mammifères, d'après des Recherches faites chez le Lapin.' Bruxelles.

such a work, to be taken up under the stimulus of present developmental questions, could not fail to be of great use; and he accordingly commenced observations on the ova of rabbits. The present small publication is the forerunner of a larger memoir which is to contain numerous plates.

According to Van Beneden's observations the germinal vesicle of the rabbit's ovum includes a clear liquid which is traversed by a granulous substance (nucleoplasma) often in the form of a reticulum. In addition to this there exists a nucleolus and a few round bodies called pseudo-nucleoli.

As maturity approaches the germinal vesicle moves towards the periphery of the ovum, which has become distinguishable into cortical and medullary portions, and takes on an ellipsoid form applying itself to the pellucid zone. A clear, non-granular portion of the cortex then accumulates about the germinal vesicle, forming what Van Beneden calls *la lentille cicatriculaire*. Meanwhile the nucleolus fuses with that part of the vesicular membrane which is nearest the edge of the ovum, and the membrane itself, becoming thinner, gradually disappears.

While this is taking place the nucleoplasma together with the pseudo-nucleoli melt into a granular *nucleoplasmatic body*, and the fluid contents of the germinal vesicle are absorbed into the cicatricular protoplasm. Simultaneously the changed nucleolus, which, on fusion, had extended into a plate, gathers itself together into an ellipsoidal or lenticular *nucleolar body*. By the time the vesicular membrane has disappeared the cicatricular protoplasm, after infiltration by the vesicular fluid contents, has become again granular and indistinguishable from the surrounding cortical mass; and the vitellus commences to withdraw from contact with the pellucid zone and to express into the space thus left a clear *perivitelline fluid*. Into this perivitelline space the nucleoplasmatic and nucleolar bodies are expelled, forming the *Richtungsbläschen* (Fritz Müller) or *globules polaires* (Robin) of other observers. All distinction of cortex and medulla has by this time vanished, and the yolk, at this stage, truly merits the name *Monerula* (Haeckel).

The ovum is now ready for fertilisation; all the steps hitherto recorded taking place while the ovum is still ovarial.

The act of fertilisation is the passage of spermatozoids through the *zona pellucida*, which Beneden is now convinced presents no micropyle; once in the perivitelline space, they apply themselves by their heads firmly to the vitelline sphere, without, however, in any case penetrating into its substance.

Fertilisation is, therefore, the fusion of the spermatic substance with the superficial layer of the yolk.

After its occurrence the yolk is divisible into three zones, a central finely and regularly granular, an intermediate coarsely and irregularly granular, and a superficial of highly refractile, punctate appearance. In the last-mentioned layer a small, round, homogeneous spot—*the peripheral pronucleus*—developes, resembling somewhat a vacuole, and not tinting in perosmic acid like the surrounding substance. It increases in size and obtains interior nucleolar structures.

At the same time two or three small clear irregular bodies appear in the central zone and fuse into a *central pronucleus* of somewhat indefinite contour and knobbed or knotted surface (un corps bosselé à sa surface).

The pronuclei approach and meet near the centre of the ovum, the protoplasm of which exhibits a radiate arrangement. One of them—the peripheral—continues to grow, while the other diminishes in size; until finally but one body can be seen, of irregular and indistinct contour and containing no nucleoli. The exact mode of formation of this nucleus—whether by fusion of both pronuclei, or by absorption of one by the other—Beneden could not clearly determine.

The origin of the peripheral pronucleus from that layer which was immediately touched by the spermatozoids gives colour to an hypothesis of a fusion of male and female elements in the union of the pronuclei; but van Beneden leaves it for the present among the number of mere possibilities.

The changes which occur immediately after this stage have not yet been studied by van Beneden as closely as they deserve to be. Nevertheless, he saw the primary cleavage nucleus elongate and a figure develop which he compares with Auerbach's karyolytic figure; and he believes, moreover, that he has sufficient ground for stating that the vacuoles which the latter observer describes as occurring in the intermediate band are merely remains of the primary embryonal nucleus, *i. e.* the nucleus of the primary cleavage mass, and, as such, are capable of becoming tinted by treatment with picrocarminate of ammonia.

Immediately after completed cleavage, each segment is of regular spherical shape, and presents a clear spot within, which, under a high power of microscope, is seen to be composed of two distinct parts—a smaller, round part, considered by van Beneden to be derived from the primary embryonal nucleus and called by him the *derived pronucleus*, and a larger part, with knobbed surface, incompletely enveloping the former, called the *produced pronucleus*

(*pronucleus engendré*). The latter is the remnant of the clear substance which had accumulated at the poles of the spindle-shaped nucleus in the primary cleavage mass, and, being merely differentiated protoplasm of the cell-body, it has no genetic connection with that nucleus. The derived pronucleus increases at the expense of the produced pronucleus until the latter is all absorbed, when the former acquires nucleoli and constitutes the nucleus of the segment.

At this point van Beneden's research ceases to have special interest for us now; a detailed account of what follows need not, therefore, be given. To be brief, certain differences in size, microchemical reaction, and subsequent development presented by the cleavage segments of the second order, led him to apply the terms *ectodermic* and *endodermic* to the larger and smaller masses respectively. Of the masses of subsequent orders, those derived from the ectodermic segment divide more rapidly, and in such a manner as to envelope as a cortex those derived from the endodermic segment. The cortex is provided with an aperture, called by Ray Lankester a *blastopore*, through which is extruded an endodermic stopper; and the whole structure is designated *Metagastrula*.

In conclusion, van Beneden, after describing the formation of the blastodermic vesicle (germ-vesicle), gives a brief *résumé* of the results of his researches on the multiplication of the cells of the ectoderm in rabbits—results which are almost identical with those of Strasburger. The elongating nucleus becomes indistinct and irregular in margin. The nucleoli disappear; the contents separate into a clear *suc nucléaire*, indifferent to staining fluids, which collects at the extremities, and a homogeneous *essence nucléaire*, tinging deeply in carmine, which collects in the middle as an equatorial plate or disc of highly refringent, ovoid granules. At this stage, it is to be remarked, Beneden never saw the longitudinal striation remarked by Strasburger and others.

A finely granular mass now collects about the poles of the nucleus—a mass which is possibly the same as that of the *pronucleus engendré* spoken of before; and the protoplasm of the cell presents the appearance of stars, indicating clearly an attraction exerted by the nuclear poles. The equatorial plate splits into two discs which move apart, being merely connected by a few threads stretched between their opposing faces. After a time, however, the threads are retracted into the discs.

The discs reach the poles and finally become the young nuclei. The intermediate clear tract differentiates at its

centre into a substance which originates a partition between the coming segments, and thus corresponds to Strasburger's *Zellplatte.*

With the preceding *résumés* before us, it is not difficult to frame a general account of the earliest stages of cleavage in which all the observers may agree.

As the ovum approaches maturity, shortly before or about the period of fertilisation, certain changes of regressive character overtake the germinal vesicle, which result in its total or partial destruction.

Under the influence of the fertilising act the ovum again becomes nucleated, the nucleus arising in a fusion of pronuclear bodies, one of which may possibly be derived from the fertilising element. The nucleus at once begins a cycle of changes about the facts of which the unanimity of the various observers leaves us no doubt. It first looses its nuclei and elongates into a spindle, which becomes indistinct and irregular, due probably to the collection about it of a clear or finely granular substance. In the plane of its equator a granular disc or zone of matter more receptive of coloration by staining fluids appears and splits, after a short time, into two parts, each of which begins a movement towards that pole of the spindle which is nearer to it. At the same time, or a little while before, the granular cell-body exhibits in its substance a distinct radiation as of two stellate figures which centre about the tips of the nucleus.

The two disc-segments gain at length the extremities of the spindle, and a striation of meridian lines is seen to extend between them. Again, in the equatorial plane, a differentiation of structure occurs, and a second granular plate is formed The disc-segments are the nuclei of the new cleavage-spheres, and the second equatorial plate marks out, and assists in, division of the cell-mass, which has meauwhile been progressing inwards from the equator.

With regard to the germinal vesicle, both Auerbach and Strasburger agree with the numerous observers who describe it as disappearing entirely either before or during fertilisation; but neither gives in detail an account of its destruction. Hertwig and van Beneden, on the other hand, have entered into the question fully, and have arrived at very different conclusions. Their descriptions of the germinal vesicle in the unripe ova of *Toxopneustes lividus* and the rabbit agree in all particulars—an agreement which is noteworthy in regard to the granulous reticular threads of protoplasm described as stretching across the vesicle.

In their accounts of the final dissolution, however, they differ. In Hertwig's view the whole structure, after having become peripheral, disappears, leaving behind it of its contents merely the germinal spot, which remains as the nucleus of the ovum (*Eikern*). Van Beneden thinks that the walls of the peripherally-seated vesicle fuse with the nucleolus or germinal spot, and the body thus formed, together with the remains of the reticular threads and pseudo-nucleoli, which constitute another body, are rejected as so-called *Richtungsbläschen* or *corps directeurs*.

In this connection mention must be made of the statements of Balfour[1] in a monograph on the developmental history of Elasmobranch Fishes, which he is now publishing. He believes that the vesicle atrophies as the ovum ripens, and that its contents become indistinguishable from the surrounding protoplasm. The membrane, in the cases where it is thick and resistant, may escape complete absorption and be extruded bodily, as in the instances of osseous and elasmobranch fishes, birds, &c.; but in the majority of ova it is quite absorbed, although it may appear again in the guise of the *Richtungs-Körper*.

The fate of the germinal vesicle has always excited great interest among embryologists, and three different views exist concerning it. According to the first, which, as we have seen, is shared by Auerbach and Strasburger, the vesicle disappears entirely. Purkinje[2] and von Baer[3] both describe it as moving towards the periphery of the ovum while still in the oviduct, and as finally becoming ruptured and disappearing; and in this description they have been followed by many observers in more recent times.

According to the second view, the germinal vesicle continues as such, and undergoes division prior to that of the whole cell. This, which is supported in special cases by statements of Joh. Müller, Leydig, Gegenbauer, and Fol,[4] was maintained by van Beneden[5] for the whole animal king-

[1] In the 'Journal of Anatomy and Physiology,' January, 1876.

[2] Purkinje: 'Symbolæ ad ovi avium historiam ante incubationem.'

[3] C. E. v. Baer: 'Untersuch. ü. die Entwicklungsges. der Fische,' 1835. C. E. v. Baer: 'Untersuch. ü. die Entwicklungsges. der Thiere,' Bd. ii.

[4] Joh. Müller: "Ueber die Erzeugng von Schnecken u. Holothurien," 'Archiv f. Anat. ü. Phy.,' 1852. Leydig: "Ueber den Bau u. die Systematische Stellung der Räderthiere," 'Zeitsch. f. Wiss. Zool.,' Bd. vi. Gegenbauer: "Zur Lehre vom Generationswechsel u. der Fortpflanzung bei Medusen u. Polypen," 'Untersuch. über Pteropoden u. Heteropoden.' Fol: "Die Erste Entwicklung des Geryonidenneies," Jena. 'Zeitsch. f. Med. u. Naturwiss.,' Bd. vii.

[5] Van Beneden: 'Recherches sur la Composition et la Signification de l'œuf,' Bruxelles, 1870.

dom. The last-mentioned writer supposed that the germinal vesicle is in no case abolished, but merely rendered for a time invisible by reason of certain changes in the surrounding yolk, and that it reappears before cleavage.

According to the third view, of which Hertwig, among the authors whose views we have been examining, is the representative, the germinal spot is saved in the degeneration of the germinal vesicle and appears as the nucleus of the ovum ready for the changes which fertilisation will bring on. This view of the continuation of the germinal spot is not new. Derbès,[1] in 1847, described the ovarial ovum as consisting of three zones, the germinal spot, the germinal vesicle, and the yolk; of which the middle one afterwards disappears. Von Baer,[2] again, in the case of echinoderms, states that the germinal spot remains as a nucleus to the ovum when the germinal vesicle is no longer to be seen. Similar statements are also made by Leydig[3] concerning *Piscicola*, and by Bischoff[4] in the case of mammals.

With regard to these views it seems impossible, as Hertwig points out, to doubt the correctness of those who assert a disappearance of the germinal vesicle. To suppose that such a conspicuous object as the germinal vesicle was overlooked by them, becomes extremely difficult when the number and trustworthiness of the observers is considered. Hence it must be concluded that the error lies with those who have believed in its continuance; for it is scarcely possible that both sets of statements can be correct in the particular cases to which they refer; or, in other words, that such a marked difference should exist between, let us say, Hydra on the one hand (where the germinal vesicle disappears) and Medusæ (where the vesicle divides according to Gegenbauer) on the other. The possibility of such an error is by no means remote; for the descriptions of the vesicle as a homogeneous non-nucleolated vacuole, which are given by the majority of the observers, are such as to suggest strongly a confusion with the nucleus of the primary cleavage mass, which is acknowledged by all to undergo division.

The view of a continuance of the germinal spot alone admits of no such objection. Those who, like Auerbach and

[1] Derbès: "Observ. sur le Mécanisme et les Phén. qui accompagnent la Formation de l'Embryon chez l'Oursin comestible," 'Ann. des sc. nat. Zoologie,' 1847, tome viii.

[2] C. E. v. Baer: "Neue Untersuch. ü. die Entwicklung der Thiere," 'Froriep's Neue Notizen,' Bd. 39.

[3] Leydig: "Zur Anatomie von Piscicola Geometrica," 'Zeitsch. f. wiss. Zool., Bd. i.

[4] Bischoff: "Entwicklungsgesch. des Kaninchencies," 1842.

Strasburger, believe that the germinal vesicle vanishes entirely, necessarily suppose the existence of an enuclear stage in the development of the first cleavage mass. But on this point Hertwig thinks there is room for doubt.

The difficulty of distinguishing in the fresh condition a small body like the original germinal spot, with refractive powers but slightly different from those of the protoplasmic mass which surrounds it, has been much underrated. Until, therefore, further attempts to differentiate a nucleus or interior body by means of staining fluids have led to similar negative results, this criticism must be allowed to have weight.

The statements as to the origin of the primary cleavage nucleus are those which present the points of greatest difference among the authors who have recently investigated the matter. Strasburger's view is, however, singular; for he connects the nucleus in question with the skin or cortical layer of protoplasm of the ovum, by direct descent. This he does without putting forward any strong reasons for such a relationship, and in spite of the fact that the nucleus stains much more deeply than any other part of the ovum when treated with colouring fluids—a circumstance which Hertwig suggests as indicating a distinction of substance.

The rest describe a fusion of pronuclei, generally of two, but sometimes of more than two.[1] To each of these pronuclei Auerbach ascribes a similar origin, viz. the vacuolation of the cell-body and the collection into the vacuoles of a fluid nuclear matter. Both Hertwig and van Beneden speak of the pronuclei as bodies staining in colouring fluids, and having other nuclear characteristics; and both regard one of the pronuclei (Hertwig's *Spermakern*, van Beneden's *pronucleus périphérique*) as possibly in some way connected with the fertilising element,—indeed, Hertwig believes it to be the head or nucleus of the spermatozoid.

In the subsequent history of the nucleus, as has already been said, there is greater agreement. The discrepancies between the account of Auerbach and those of Strasburger, Hertwig, and Van Beneden, or, more correctly speaking, the incompleteness of the former as compared with the latter, is entirely explained by the respective methods of observation adopted; since Hertwig, when he examined recent specimens only, overlooked the interior nuclear changes so fully verified by Strasburger—an oversight which he repaired by a study of

[1] Bütschli.

the same objects hardened and carminized. Differences of hypothesis could hardly be avoided.

Although it required the perfection of modern instruments and technical methods to furnish a complete history of cell-division, yet certain stages of it had already been dimly or partially seen by earlier observers. Thus Bagge,[1] Gabriel,[2] and Kölliker[3] have figured appearances similar to those shown in Plate I (figs. 5—7), which were thought to be due to the division of the central nucleus into two. Moreover, the first-mentioned author describes an elongation of nucleus which resulted in the formation of a finger-biscuit-shaped mass, and ultimately in its division into two parts. Again, the radiate arrangement of the granules in the protoplasmic cell-mass has been from time to time noticed as accompanying cleavage, viz. by Derbès[4] in the case of Echinoderms, and by Krohn[5], Kowalewsky,[6] and Kupffer[7] in Ascidians.

Of the changes affecting the nucleus during its elongation Auerbach saw nothing but the collection of a clear, stellate mass at its tips, which he thought to be due to the expulsion of its contents, and an appearance as of two movable vacuoles in the intermediate band. Among the rest the chief point of distinction is that Hertwig describes no structure equivalent to the cell-plate of Strasburger and Van Beneden, which assists in the partition of the whole cell. It is important to add, however, that Balfour,[8] in the fusiform nuclear structures which he describes as taking part in cell-division under certain circumstances, represents an equatorial double row of granules, between which the line of division of the cell undoubtedly passed.

The difference of opinion as to the origin of the nuclei of the new segments is very well marked. Auerbach, as we have already seen, regards their origin as palingenetic: the nuclear substance, dispersed for a time throughout the cell-mass, is re-collected into the vacuoles, which will become the young nuclei. Strasburger and van Beneden are equally explicit. According to them, the two halves of the nuclear disc or *essence nucléaire* move to the extremes of the elon-

[1] Bagge, 'Diss. inaug. de Evolutione Strongyli, &c.,' Erlangen, 1841.
[2] Gabriel, 'De Cuculiani Elegantis Evolutione,' Berolini, 1853.
[3] Kölliker, 'Müller's Archiv,' 1843.
[4] Derbès, loc. cit.
[5] Krohn, "Ueber die Entwick. der Ascidien," 'Archiv f. Anat. u. Phys.,' 1852.
[6] Kowalewsky, 'Mém. de l'Acad. imp. de St. Petersburg,' tome x.
[7] Kupffer, "Die Stammesverwandtschaft zwichen Ascidien u. Wirbelthieren," 'Archiv f. Mikro. Anat.,' Bd. vi.
[8] F. M. Balfour, loc. cit.

gated parent-structure and become at length the daughter-nuclei, increasing, as van Beneden asserts, at the expense of the substance which has already collected about the tips of the original nucleus.

Although Hertwig in his hardened and stained specimens does not certainly speak of the derivation of the young nuclei from the first *median thickened zones*, there can hardly be a doubt that the *lateral thickened zone* (which afterwards became the nuclei) correspond entirely to the segments of the nuclear disc described by Bütschli, Strasburger, and Beneden, and resulted from division of the former zone.

In a paper in which the names of so many histologists have been mentioned as in this, it would be unfair to omit a reference to the researches of two observers who were among the first to note certain of the new phenomena. Fol[1] and Flemming[2] have described the appearance of two stellate figures, which the former calls 'centres of attraction,' in cells which are about to divide. Between these for a time certain structures, supposed by them to be the remnants of the old nucleus, are demonstrable by means of reagents (Fol used acetic acid, Flemming carmine). During and after division of the whole cell the new nuclei develope either in the fusion of several vacuoles in the centre of the star-like figures (Fol), or as new carminizable structures which appear in the same situation (Flemming). It is hard to resist the conclusion that the intermediate structures seen by Fol and Flemming were the same with those described by others as portions of the dividing primary nucleus.

The researches which have been considered represent an important step in the progress of histological inquiry —a step which now awaits the confirmation of a more numerous series of observations.

[1] Fol: "Die erste Entwicklung des Geryonideneies," 'Jenaische Zeitsch. f. Med. u. Naturwiss,' Bd. vii, 1873.

[2] Flemming: "Ueber die ersten Entwicklungserscheinungen am Ei der Teichmuschel," 'Archiv. f. Mikros. Anat.,' Bd. x. "Studien in der Entwicklungsgesch. der Najaden," 'Sitzb. der K. Acad. d. Wissensch,' iii, Abth., Jahrg, 1874, Bd. lxxi.

Contributions *to the* History *of the* Germinal Vesicle, *and of the* First Embryonic Nucleus. By Edouard van Beneden, Professor in the University of Liège. (With Plate XIII.)

I have had the honour of presenting to the Academy a summary exposition of the results of my researches on the maturation of the ovule, the fecundation and earliest phenomena in the embryonic development of the rabbit, up to the moment of the appearance of the primitive streak more especially. I have made known those of my researches which establish the disappearance of the germinal vesicle, the formation of the directive bodies (*Richtungsbläschen* of Fritz Müller), and the appearance of the first embryonic nucleus. The recent publication of the researches of Auerbach,[1] Bütschli,[2] and Strasburger,[3] on the formation and division of the nuclei of cells, led me, in the course of my researches on the development of mammalia, to investigate very specially how the first nucleus of the embryo appears and how cells multiply in the embryonic laminæ. Long before I became acquainted with these researches, I had observed that the germinal vesicle of the rabbit disappears, independent of fecundation, and that the disappearance of this element is the indication of the complete maturity of the ovum.

At the very moment when I was completing the drawing up of my preliminary communication[4] on the maturation of the egg, its fecundation and development in the rabbit, there appeared in Germany an important memoir by Oscar Hertwig[5] on the formation, fecundation, and division of the egg of an echinoderm belonging to the order Echinida, *Toxopneustes lividus*. M. Hertwig and I, endeavouring to solve the same problems, but having chosen for our researches animals belonging to different groups, have arrived at

[1] Leopold Auerbach: 'Organologische Studien,' Breslau, 1874.

[2] O. Bütschli: "Vorläufige Mittheilung über Untersuchungen betreffend die ersten Entwickelungsvorgänge im Befruchteten Ei von Nematoden und Schnecken," 'Zeitsch. für Wiss. Zoologie,' Bd. xxv. O. Bütschli: "Vorläufige Mittheilung einiger Resultate von Studien über die Conjugation der Infusorien und die Zelltheilung," 'Zeitsch. für Wiss. Zoologie,' Bd. xxv.

[3] Eduard Strasburger: 'Ueber Zellbildung und Zelltheilung,' Jena, 1875.

[4] Edouard van Beneden, "Le maturation de l'œuf, la fécondation et les Premières Phases du Developement Embryonnaire des Mammifères d'apres des recherches faites chez le Lapin," (Communication preliminaire), 'Bull. de l'Acad. Roy. de Belgique,' 2e série, t. xl, 1875.

[5] Oscar Hertwig, "Beiträge zur Kenntniss der Bildung, Befruchtung, und Theilung des Thierischen Eies," 'Morphologisches Jahrbuch von C. Gegenbauer.'

identical conclusions on certain questions of capital importance, and at very different results with regard to other, equally fundamental points.

Among the problems which he and I have solved very differently are, in the first place, the history of the germinal vesicle; and secondly, the question of the formation of the first embryonic nucleus.

My researches on the ovum of the rabbit have proved to me that no morphological part of the germinal vesicle is found in the yolk at the moment of fecundation. The nucleolus united with the substance which constituted the membrane of the vesicle is eliminated to form one of the "directive bodies;" the nucleoplast with the pseudo-nucleoli are thrown off into the perivitelline liquid, to form there the second polar globule. The liquid of the vesicle remains in the yolk, and becomes confounded with the cortical substance of the ovum, which from this moment is no longer distinguishable from the medullary substance. There cannot then be, in the rabbit, any genetic connection between the germinal vesicle or one of its parts, and the embryonic nucleus which appears in the egg after fecundation. I have moreover been able to observe all the phases in the formation of the latter. The first nucleus is developed at the expense of a body formed in the cortical layer of the ovum, which I have called the *peripheral pronucleus*, and of another body which appears in the centre of the yolk, and which I have called the *central pronucleus*. It is probable that the first embryonic nucleus is not formed by the fusion of the two pronuclei:—the peripheral pronucleus, at first smaller than the other, enlarges at the expense of the central. The latter becomes attached to the former, and then its substance becomes absorbed by it, as, I think, by a process of endosmosis.

According to the observations of M. Hertwig on the *Toxopneustes lividus*, things take place in a different way. When the germinal vesicle has quitted the centre of the ovum to place itself under the membrane, and has, so to speak, left the yolk, the germinal spot in its turn forsakes the germinal vesicle, penetrates into the yolk, and becomes what the author calls the nucleus of the ovum (*Eikern*); the germinal vesicle then undergoes retrograde metamorphosis; its membrane becomes dissolved, and the rest is finally absorbed by the yolk.

As to the formation of the first nucleus of the embryo, which he calls *nucleus of the first cleavage-sphere*, or more simply, *cleavage nucleus*, M. Hertwig has ascertained that it is the product of the copulation of two nuclei. In from five to ten minutes after the sperm has been mixed with the eggs,

there appears near the surface of them a small homogeneous body. This element, which appears like a spot or a clear space, is formed by a mass of protoplasm, devoid of granulations. This space enlarges slightly and becomes the centre of a radiated figure, a little sun, from which rays, the length of which gradually increases, spread out in every direction. These rays are nothing but lines along which the yolk-granules are arranged. If the clear space is carefully examined, it is seen to contain a small homogeneous corpuscle. This has nearly the same refractive power as the surrounding protoplasm which makes it a little difficult to see. Sometimes Hertwig has seen a delicate line start from this corpuscle, pass to the periphery of the egg, and become continued into a little filament floating in the perivitelline liquid. The clear spot changes its place; it gradually approaches the centre of the yolk and goes to meet the nucleus of the egg, which also reaches the centre of the vitelline sphere. The two bodies finally meet near the middle of the egg. The homogeneous corpuscle contained in the clear space which comes from the periphery appears to be formed of the same substance as the nucleus of the egg, and is, like that, coloured by carmine. Hertwig calls it a small nucleus—measuring 4μ, while the nucleus of the egg is not less than 13μ. The nucleus of the egg changes its shape, executes amœboid movements, grows, and is soon surrounded by the clear protoplasmic substance which comes from the periphery; finally, it becomes fused with the little nucleus and from the fusion of these two nuclei arises the first cleavage-nucleus. While these last modifications are being accomplished, the radiated figure remains; it even extends, and becomes still more clearly marked; it involves all the yolk. The nucleus of the egg, and the little peripheral nucleus which becomes attached to it, both surrounded by a layer of transparent protoplasm without granulations, occupy the centre of the stellate figure.

Since the clear space near the periphery of the egg appears constantly five or ten minutes after the eggs have been mixed with the spermatic fluid, Hertwig does not hesitate to consider the formation of this space as the result of fecundation. The small homogeneous body which he has found to exist there is the head of a spermatozoon; the filament which starts from it is the tail of the same The head of the spermatozoon is one of the two nuclei which meet and conjugate; for this reason Hertwig calls it the *spermatic nucleus* (Spermakern). *The first cleavage-nucleus is then the product of the fusion of the nucleus of the egg* (Eikern), *which is only the original germinal spot, with the spermatic nucleus* (Sperma-

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It is plain that there is in certain respects a remarkable agreement between Hertwig's observations on *Toxopneustes* and my researches on the rabbit. We have both ascertained (1.) that there appears near the surface of the egg a clear space, which I have called, in order not to prejudge in any way its significance, the *peripheral pronucleus*. This peripheral nucleus I have regarded, as at least in part, formed of spermatic substance. (2.) This superficial nucleus penetrates into the yolk and goes to meet another transparent body, of which the character and significance are different from those of the peripheral nucleus; and which I have called the *central pronucleus* in contradistinction to the peripheral pronucleus. Hertwig calls it *nucleus of the egg*, in opposition to his *spermatic nucleus*.

(3.) We have both ascertained that the nucleus of the first cleavage-sphere, called by me *first embryonic nucleus*, by Hertwig *cleavage-nucleus*, is developed at the expense of the two nuclear elements after they have joined and become united in the centre of the yolk. We have both supposed that the formation of the first embryonic nucleus results from the union of a male element and a female element; and although I have not applied the term conjugation to the fact which essentially characterises the formation of the first nucleus, still the idea was not the less present in my mind, at least in a hypothetical form.

The facts as to which we are in complete disagreement are two in number. (1.) According to Hertwig, the germinal spot does not disappear—it becomes the nucleus of the egg (*Eikern*). According to my observations there exists no genetic bond between the central pronucleus (*nucleus of the egg* of Hertwig) and the germinal vesicle or any of its parts; the central pronucleus which appears after fecundation is an element of new formation. (2.) According to Hertwig, the peripheral nucleus—the *Spermakern*—is the head of a spermatozoon, and the transparent material surrounding it is protoplasm without granulations. In my opinion the clear space which appears in the cortical layer of the egg is a nuclear body (the peripheral pronucleus); the refractive corpuscles which appear on the spot are nucleolar elements. I propose in the following pages to criticise the opinions expressed by Hertwig, and to make known the observations which I have had the opportunity of making on the germinal vesicle of the egg of an echinoderm belonging to the order Asterida; *Asteracanthion rubens*.

I. *Does the germinal spot disappear or does it remain so as to become the central pronucleus* (*Eikern of Hertwig*) ?

Hertwig expresses the opinion that the germinal vesicle disappears in *Toxopneustes lividus ;* but he believes that the germinal spot (body or spot of Wagner) remains so as to become his (nucleus of the egg). He has not been able to arrive at a direct proof of this proposition ; his opinion rests upon indirect proofs, of which I will give a summary.

(1.) The nucleus of the egg has the same dimensions (13 μ) as Wagner's germinal spot; both are corpuscles without any membrane and formed of tolerably firm and homogeneous substance.

(2.) Like the substance of Wagner's spot, the nucleus of the egg is coagulated by osmic acid and coloured more deeply black than the yolk. Both are stained red by carmine. When acted on by acetic acid and chromic acid the two elements undergo a sort of superficial coagulation which produces a finely granular cortical layer, and some spots also granular, in the interior.

(3.) We never observe the nucleus of the egg and the germinal spot at the same moment in the same egg. So long as the germinal spot is seen in the germinal vesicle, when the latter has become superficial and lenticular, it is impossible to discover a nucleus in the yolk. From the time when this nucleus exists the germinal vesicle is destitute of its spot. The two elements are never wanting at the same time.

(4.) Hertwig has never succeeded in tracing any alteration in Wagner's spot, even in those eggs where the germinal vesicle was undergoing retrograde metamorphosis. " Fur die Annahme, dass der Keimfleck, wie das Keimbläschen zerfällt, lässt sich daher keine directe Beobachtung anführen." He was equally unable to observe a new formation of the nucleus of the egg.

(5.) The nucleus of the egg at the moment when it appears is situated near the germinal vesicle ; the Wagner's spot at the moment of its disappearance is adjacent to the yolk.

Hertwig has not then observed directly the transformation of Wagner's spot into the body which he calls the nucleus of the egg ; *he has never seen Wagner's body leave the germinal vesicle in order to penetrate into the yolk.* Hence some doubt must remain as to the identity of these two elements, whatever may be the arguments by which he seeks to establish this conclusion. Hertwig himself acknowledges this fact when he writes : " Bei Abwägung aller dieser Verhältnisse

kann zwar die Möglichkeit dass der Keimfleck sich auflöst und der Eikern neu entsteht, solange nicht der directe Uebergang des ersteren in den letzteren beobachtet ist, nicht ganz von der Hand gewiesen werden."

We may, moreover, make the following remarks on the arguments adduced by Hertwig:

1. Proofs drawn from the physical and microchemical characters of the nucleus of the egg can have only a secondary value; but the characters common to this structure and the spot of Wagner belong to all young nuclei. The only conclusion from these facts is then that the nucleus of the egg is a young nucleus.

2. It does not necessarily follow from our never finding the germinal spot and the nucleus of the egg existing at the same moment that one is a transformation of the other; the disappearance of the nucleolus coincides with the appearance of the nucleus of the egg; but does it follow that the one fact is caused by the other? Nevertheless, the coincidence is surprising; according to my observations on the Mammalia, the formation of the central pronucleus is subsequent to fecundation, and happens, consequently, long after the disappearance of the germinal vesicle.

3. I was much surprised, after reading Hertwig's memoir, to find no mention there of the "directive bodies" (Richtungs-bläschen) which have been seen in many Echinoderms, and which cannot fail to occur also in *Toxopneustes*. It would have been interesting to know how these elements are formed in Echinodermata, since, according to my observations on the Rabbit, one of these bodies is nothing else than the germinal spot thrown off into the perivitelline liquid after its previous fusion with the membrane of the germinal vesicle.

4. I cannot pass over so easily as Hertwig does the differences between the appearance presented by the nucleus and the characters of the germinal spot. For my own part, I do not believe that the nature of the media in which these elements are respectively observed can account for the marked differences which, according to Hertwig's drawings, exist between Wagner's spot and the nucleus of the egg. This opinion is founded upon observations which will be related further on.

5. Hertwig has never seen any modifications produced in the characters of Wagner's body during the retrograde metamorphosis of the germinal vesicle. In this I have been more fortunate, not that I have studied the eggs of Toxopneustes, but I made some observations eighteen months ago

on the germinal vesicle in an echinoderm of our coasts, *Asteracanthion rubens*. The result of my researches is that, in this animal, Wagner's spot disappears in the germinal vesicle before the characters of the latter are altered. It cannot be supposed that in the starfish Wagner's spot undergoes progressive modifications which bring about its complete dissolution in the germinal vesicle, and that this element becomes in *Toxopneustes lividus* one of the first constituent parts of the first cleavage nucleus. If M. Hertwig has not seen the germinal spot undergo any modifications, we need not conclude, I think, that such modifications do not occur. His own figures, indeed, will I think show that in *Toxopneustes* Wagner's spot undergoes the same modifications as in the starfish.

Before relating my observations on the disappearance of the germinal vesicle in *Asteracanthion rubens*, I must state shortly in what circumstances, for what reasons, and with what ideas I undertook these researches. When I presented to the Academy my memoir on the composition and significance of the egg, I was convinced that the germinal vesicle does not disappear, but that it divides, after fecundation, so as to produce the nuclei of the two first cleavage-spheres.[1]

Up to the time when Johann Müller published his researches on *Entoconcha mirabilis*[2] no one had any doubts as to the disappearance of the germinal vesicle. Leuckart, in his article "Zeugung," published in Wagner's Handwörterbuch, expressed as follows the conclusion which he felt authorised to draw from a comparison of all the researches made on this subject.[3]

"Fassen wir alle diese Thatsachen zusammen, dann kann es wirklich kaum noch zweifelhaft bleiben dass das Verschwinden des Keimbläschens einen Vorgang bezeichnet, der mehr der Bildungsgeschichte des Eies, als der Entwickelungsgeschichte des späteren Embryo zugehört. Das Einzige, was der Aufbau eines neuen Thieres voraussetzt, ist die Anwesenheit eines entwickelungsfähigen Materiales."

"If we put all these facts together, there can indeed hardly remain any doubt that the disappearance of the germinal vesicle indicates a process belonging more to the history of the formation of the egg than to the later history of develop-

[1] "Recherches sur la Composition et la Signification de l'œuf basées sur l'Étude de son mode de Formation et des Premiers Phénomènes Embryonnaires," 'Mém. Couronné de l'Acad. Roy. de Belgique,' T. xxxiv.

[2] Joh. Müller, "Ueber Synapta Digitata und über die Erzeugung von Schnecken in Holothurien," Berlin, 1852, p. 17.

[3] "Handwörterbuch der Physiologie von R. Wagner," 'Art. Zeugung von R. Leuckart,' p. 922.

ment. The only essential condition of the construction of a new animal is the presence of a material capable of development."

This was at that time the opinion of most, if not of all naturalists. But so great was the authority of Johann Müller, so complete the confidence which was placed in his observations and conclusions, that the opinion he promulgated on the permanence and division of the germinal vesicle in *Entoconcha* caused all the affirmative statements made in previous works to be called in question.

Shortly afterwards Leydig[1] announced that he had confirmed in the Rotifera the results obtained by Müller in his researches on the development of *Entoconcha*. Mecznikow[2] observed the division of the germinal vesicle in the Coecidomyæ and Aphides. "It is hardly possible," he writes, "to call in question the generality of this fact in insects." Pagenstecher[3] made the same observation in the Trichinæ; Leuckart[4] in the Oxyurides; Keferstein[5] in *Leptoplana tremellaris;* Gegenbauer[6] in the Medusæ, Siphonophora (Corynidæ, Calycophoridæ, Physophoridæ), Pteropoda, Heteropoda, and finally in Sagitta. Häckel[7] and Kölliker[8] confirmed as regards the Siphonophora the statements of Gegenbauer. Finally, I myself observed the division of the germinal vesicle in the transparent and easily observed egg of *Distoma Cignoides*.[9] Resting as much on my personal observations as on those of the eminent naturalists just quoted, I expressed in a hesitating form the opinion already announced in a manner equally general by Leydig,[10] that the disappearance of the germinal vesicle is only apparent, and that the embryonic development begins with the division of

[1] Fr. Leydig, "Ueber den Bau und die Systematische Stellung der Räderthiere," 'Zeit. für Wiss. Zoologie,' Bd. vi, p. 102.

[2] Mecznikow, "Embryologische Studien an Insecten," 'Zeitsch. für Wiss. Zool.,' Bd. xvi, p. 484.

[3] Pagenstecher, 'Die Trichinen,' Leipzig, 1865.

[4] Leuckart, 'Die Menschlichen Parasiten,' Bd. ii, 2e Lief., p. 322.

[5] Keferstein, 'Beiträge zur Anatomie und Entwickelungsgeschichte einiger Seeplanarien,' Gottingen, 1868.

[6] Gegenbauer, "Beiträge zur Näheren Kenntniss der Siphonophoren," 'Zeit. für Wiss. Zool.,' Bd. v. 'Zur Lehre vom Generationswechsel bei Medusen und Polypen,' p. 24. 'Untersuchungen über Pteropoden und Heteropoden,' Leipzig, 1855. 'Ueber die Entwickelung der Sagitta,' Halle, 1856.

[7] E. Häckel, 'Zur Entwickelungsgeschichte der Siphonophoren,' Utrecht, 1869.

[8] Kölliker, 'Die Schwimmpolypen von Messina,' Leipzig, 1853.

[9] Edouard van Beneden, loc cit., p. 30.

[10] Leydig, 'Lehrbuch der Histologie,' p. 10.

this element. I expressed myself in the following words: "En résumé, je considére non comme demontré, mais comme très-probable, que la vésicule germinative se divise au lieu de disparaître" (loc. cit., p. 244).

I supported this proposition by various more or less plausible considerations, and in particular, I pointed out that the disappearance of the vesicle had never been observed, no direct proof of this disappearance having been given.

I should add that the contrary proposition did not, any more than this, rest upon direct observation; no one of the authors who maintained the permanence of the vesicle rested his opinion upon any other considerations than upon a negative fact. *The vesicle is asserted not to disappear because no egg has ever been observed entirely devoid of a nucleus.*

Some observers have been inclined to trace a genetic bond between the nucleus of the impregnated egg and the 'spot' of Wagner. Leydig[1] has expressed this opinion so far as regards the eggs of Piscicola; von Baer[2] with respect to the development of a sea urchin. Bischoff[3] derives from the germinal spot of the rabbit, not only the directive bodies but also the nucleus which is found in the egg after impregnation. But Bischoff confesses that it is in his eyes, a pure hypothesis; he moreover, abandoned it shortly afterwards,[4] and in his later works, has declared it to be untenable. Quite recently, Fol[5] has found in the ripe egg of the Medusæ a nucleus, with respect to which he feels some doubt, not knowing whether it is the modified germinal vesicle or the spot of Wagner. Finally, Hertwig has just given new credit to this tottering opinion by the publication of his recent memoir, which cannot fail to produce a great effect.

A short time after the publication of my memoir on the composition and significance of the egg, two publications appeared in Germany, which are of the highest importance with respect to the question now occupying our attention.

Oellacher[6] established the fact that in the matured ovum of the salmon the germinal vesicle reaches the surface of the germ and opens itself into the space which exists at that

[1] Leydig, "Zur Anatomie von Piscicola geometrica," 'Zeit. für Wiss. Zool.,' Bd. i.

[2] K. E. von Baer, "Neue Untersuchungen über die Entwickelung der Thiere," 'Froriep's Neue Notizen,' Bd. xxxix, p. 38.

[3] Bischoff, 'Entwickelungsgeschichte des Kaninchencies,' 1842.

[4] Bischoff, 'Entwickelungsgeschichte des Hundeies,' 1845.

[5] Fol, "Die erste Entwickelung des Geryonidencies," 'Jenaische Zeitschrift,' Bd. vii, p. 474.

[6] Oellacher, "Beiträge zur Geschichte des Keimbläschens im Wirbelthierei," 'Archiv. für Mikrosk. Anat.,' Bd. viii.

period between the yolk and the ovular membrane. The opening enlarges, and the membrane of the vesicle becomes gradually detached from its contents. Finally, the latter are evacuated and the membrane spreads itself out on the surface of the germ. Some observation of the same author on "The Germinal Vesicle of the Fowl" furnish a remarkable and complete confirmation of the conclusions at which the illustrious von Baer arrived fifty years ago.

Soon afterwards there appeared the beautiful researches of Kleinenberg "On the Anatomy and Development of the Fresh Water Hydra." Kleinenberg[1] thus describes the mode of disappearance of the germinal vesicle:—"About the time when the production of pseudocells is completed the germinal spot undergoes a retrograde metamorphosis. At first it loses its circular outline and becomes irregular and angular; its substance appears coagulated; then it breaks up into little fragments, and these, unless I am mistaken, finally dissolve. So long as the egg was an amœbiform body, the germinal vesicle was situated at the centre of the yolk; but from the time that the egg begins to become rounded it takes an excentric position, and approaches that pole which is turned towards the surface. It takes its situation near to the surface, and is now covered only with a thin layer of plastic material. Here it also begins to undergo a retrograde metamorphosis which ends in its complete disappearance. Its granular contents become more and more liquefied; a part of these contents escape from the membrane with the result that the latter, which had previously remained uniformly stretched, becomes collapsed so as to form a tube of generally ovoid shape, the wall of which is thickened and folded at certain points. The part of the contents which has remained in the interior breaks up into isolated shining bodies of rounded or angular form, and of very different dimensions; amongst them are scattered some drops of a fatty liquid."

Kleinenberg thinks that these bodies are composed of a fatty material, or at least that they consist of the material which results from the transformation of albuminoid substances, and which we observe in many pathological tissues, where this appearance is a sign of fatty degeneration. According to his view the germinal vesicle disappears by fatty degeneration. On one occasion Kleinenberg believed he observed an actual hole in the germinal vesicle. "If this is a normal phenomenon," says Kleinenberg, "it is possible that the contents of the vesicle escape and mix with the sur-

[1] Kleinenberg, 'Hydra,' Leipzig, 1872, p. 42.

rounding plasma." The question as to what becomes of the membrane has not as yet been resolved; but what is certain, according to Kleinenberg, is that every trace of the germinal vesicle has already disappeared long before the moment when fecundation takes place.

Thanks to the observations of Oellacher and Kleinenberg, the disappearance of the germinal vesicle was directly demonstrated; from that time it was no longer possible to maintain the persistence of the germinal vesicle in all animals, without denying the facts observed. The question then entered into a new phase. Two observers, who must certainly be reckoned amongst the most eminent of the age, had just established *the mode in which the germinal vesicle disappears*. From that time only two alternatives were further possible; either it must be admitted that the germinal vesicle does not play the same part in all animals; that it disappears in some, and that it persists and undergoes division in others; or else it must be allowed that all the observations made by Müller, Leydig, Gegenbauer, Leuckart, Pagenstecher, Mecznikow, Kölliker, Haeckel, and myself were erroneous, or, at any rate, that the conclusions drawn from the facts observed were but little in conformity with the principles of logic. I believe that the latter of these hypotheses is of the two more probable; the opinion which affirmed the permanence of the germinal vesicle rested, in fact, on negative grounds. It was affirmed that this element does not disappear, because no egg had ever been found which was entirely devoid of all central nucleus; but it does not strictly follow that because no egg had been found entirely deprived of a central nucleus, that therefore the germinal vesicle persists. The doubts which the researches of Oellacher and Kleinenberg had aroused in my mind led me to make fresh researches. In order to make fresh observations on this point it was of importance to choose eggs in which the yolk possessed in the greatest possible degree the properties of transparency and homogeneity, and it was requisite, moreover, that they should be distinguished by the dimensions of the germinal vesicle and the (germinal) spot of Wagner. The ova of the Echinodermata, and in particular those of the *Asteracanthion rubens* realise these conditions in the highest degree. At the end of April, 1874, I betook myself to Ostend with the object of carrying out the artificial fecundation of these ova.

It is not very long ago that we were still in ignorance as to whether the sea starfish are of different sexes. Tiedemann declares that that he has never found the male organs of these

animals.[1] Nothing, however, is easier than to distinguish the ovaries from the testicles. It is true that the sexual organs have the same form, the same position, and the same volume in the two sexes; when they have reached their complete development the five pairs of sexual glands extend along the entire length of the arms, and they cause a marked elevation of the skin of the side of the back, so that one can recognise, even from the external appearance, the individuals in which the sexual products have attained maturity. The most superficial microscopic examination suffices to distinguish the contents of the testicle from those of the ovaries, and one soon learns to recognise the sex, even with the naked eye; the ovaries have a yellowish or very pale brownish tint; the testicles are of a pure milk-white colour. Moreover, the lobules of the ovarian clusters are more rounded and shorter, whilst those of the male sexual gland are elongated and rather of tubular shape.

I will first describe the ovarian ovum, such as it appears when, already free in the cavity of the ovary, it has attained the dimensions of the ripe ovum, but while its germinal vesicle is still lodged in the centre of the yolk. These ova may have either an ellipsoid form, or else they may be pyriform. Their dimensions vary between ·16 by ·13 and ·19 by ·17 millimètres. They are composed of a thick and entirely homogeneous envelope, of a finely granular yolk, and of a germinal vesicle which is situated in the neighbourhood of the centre of the yolk.

Membrane.—It is still a question whether there exists around the ovum of the Asteridea a single one or two membranes; nor is anything more certain known as to the nature and signification of these envelopes. If fresh ova which have reached the dimensions of the ripe ovum, and which still have the germinal vesicle central, are examined, we can distinguish a clear zone around the yolk which has a thickness of from ·003 to ·004 millimètres. This is quite clear, transparent, and homogeneous. It is limited where in contact with the yolk by a sharply defined contour; on the outer aspect, on the contrary, its contour is pale and so slightly marked, that it requires great attention to discern it. The index of refraction of the substance which constitutes this membrane must be very similar to that of water. This substance is very soft; it appears to be a gelatinous, mucilaginous, or albuminoid body; whence the names of "Gallerthülle, Eiweisschicht, and mucilaginous layer" which

[1] Tiedemann, 'Anatomie der Röhren-Holothurie, des Pomeranzfarb. Seesternes,' &c., 1816, p. 42.

have been given to it. More recently Hoffmann[1] has called it the "*vitelline membrane.*" To see well the characters of this membrane, examination in water must be avoided. The moment it comes in contact with water it swells considerably; one may study it conveniently by placing the eggs directly in 1 per cent. solution of osmic acid, or in a concentrated solution of picric acid. It is not coloured red by the colouring matter of carmine fluids; it is partially dissolved or contracts strongly by a prolonged stay in absolute alcohol. After remaining for a certain length of time in absolute alcohol it is only a very thin membrane which immediately envelops the vitellus, and which at certain points is in immediate contact with it, whilst at other points it is separated from it, thus forming more or less regular undulations. If one examines the transparent layer in ova which have not attained their complete development, it exhibits a radiated striation which is due to the presence of pores in the form of canaliculi of extreme tenuity; it has been long known that the ova of Holothurians present the same character.

I have never observed in the ova of the sea starfish the canal which J. Müller has discovered in several Holothuria, and which has been thought to fulfil the function of a micropyle. However, in the pearshaped ova the transparent layer is oftentimes a little thinner at the tail end of the pear.

Derbès has found in the ovum of the Echinida, independent of the *mucilaginous zone*, which I have just described, a thin membrane which is immediately applied to the surface of the vitellus, and which he calls a vitelline membrane. Several authors have affirmed since Derbès, the existence of this second membrane, not only in the Echinida, but also in the Holothurida and Asterida. I have not been able to convince myself of the existence of this membrane in the egg of the *Asteracanthion rubens*.

What is the signification of the transparent layer which exists around the ovum in all the Echinodermata? What name must be given to it? It is not possible to answer these questions in the present state of our knowledge on the formation of the egg of these animals. I have reasons for believing that the membrane is not produced by the egg itself, and that its mode of formation is the same as that of the zona pellucida of mammals. If my opinion is correct, there would be grounds for designating it by the name of Chorion. As its microscopical characters have some resemblance to

[1] C. K. Hoffman, "Sur l'Anatomie des Astérides," 'Extrait des Archives Néerlandaises,' vol. ix.

those of the zona pellucida of Mammalia, we may, at least provisionally, call it by that name which has the advantage of recalling its physical characters without in any way prejudging its *equivalence* from a morphological point of view.

Vitellus.—The yolk is formed of a clear and transparent fundamental substance (protoplasm) and of feebly refracting vitelline granules held in suspension in the protoplasm. These granules are formed of a substance, the refractive index of which is but little greater than that of the vitelline protoplasm. Hence it results that the transparence of the ovum is scarcely altered by their presence. The absence from the yolk of all vesicular or globular elements and of all highly refractive substances, causes the body of the ovum to be far from the appearance of an emulsion. It is a clear, transparent and finely granular mass. This circumstance renders the eggs eminently favorable to the study of the modifications which the germinal vesicle undergoes in the egg which has reached maturity.

It is possible to distinguish in the yolk of the ova of the sea starfish two layers, or if the expression be preferred, two substances; a cortical layer, the thickness of which is nearly equal to one third of the radius of the yolk, and a medullary mass. The cortical layer is clearer and less granular than the medullary mass; it presents, moreover, a slight radiated striation which appears to me to be wanting in the medullary mass. The limit between the two constituent parts of the vitellus is not marked by a very clear line, the cortical substance of the ovum passes insensibly into the medullary substance. Yet the zone of transition is very narrow. This distinction between the two constituent substances of the ovum of the Asterida has escaped the observation of all those who have studied the sexual products of these Echinoderms. It has not been mentioned up to the present time in any animal of this division, and I am surprised that Hertwig, who has studied the ova of the Toxopneustes with so much care, should not have observed it.

Germinal vesicle.—The germinal vesicle is perfectly spherical; it is lodged in the centre of the medullary mass of the egg. It is marked out by a very sharply defined and deep line. It encloses a transparent and perfectly homogeneous liquid. If we examine the germinal vesicle whilst it is in the yolk, we perceive in this liquid a voluminous and very conspicuous germinal spot, and around it a certain number of much smaller globules which are pseudo-nucleoli. The germinal spot, or spot of Wagner, is of large

size. It is circular, and its contour is quite regular. Sometimes it appears nodulated on its surface; and lastly, at other times it is angular and altogether irregular. It is formed of a highly refractive and very brilliant substance which encloses clear vacuoles, whose number and volume vary in different eggs. The vacuoles have already been noticed by Leydig, in the eggs of Holothuria tubulosa: "Der Keimfleck des fertigen Eies ist bedeutend schärfer contourirt als das Keimbläschen, fast fettartig und zeigt ein oder mehrere Cavitaten." Hertwig has observed that in the ova of the Toxopneustes Wagner's spot contains sometimes one, sometimes several vacuoles.

In the ova which I am describing, I have not observed amœboid movements performed by the nucleolus. Long ago de la Valette[1] had noted the fact that in the ovum of a Libellula the germinal spot might be seen to change its form and place. Mecznikow[2] has seen, not only in the spot of Wagner of several of the lower animals, but also in the salivary cells of the larva of insects, spontaneous movements of the nucleoli. Balbiani[3] has made a similar observation with regard to the ova of spiders. Alexander Brandt[4] has observed that in the eggs of *Periplaneta orientalis* the germinal spot affects all sorts of forms, and that these changes, which are really active, must be ascribed to the contractility of the substance of the spot or nucleolus. He has remarked that under the influence of heat, these movements become so active, that it is difficult to draw the successive changes of form which are produced.

Auerbach[5] has recognised changes of form, presenting all the characters of amœboid movements, in the large nuclei of the embryonic cells of the Muscida and in the germinal spots of the ovum of the pike; Hertwig has seen the same phenomenon occur in the nuclei of the eggs of the frog and also in the germinal spot of Wagner, in the ovum of *Pterotrachea*. More than four years ago, I observed changes in form, and enlargement and diminution of size, whilst watching Wagner's spot in the germinal cells of the *Polystomum integerrimum*, and I had remarked that the diminution in volume of these nucleoli corresponded with

[1] De la Valette: "Uber den Keimfleck und die Deutung der Eitheile," 'Archiv. für Mikrosk. Anat.,' bd. ii, 1866.

[2] Mecznikow, 'Virchow's Archiv.,' bd. xii.

[3] Balbiani (quoted by Auerbach), see Keferstein. 'Jahresbericht,' für 1865.

[4] Alexander Brandt, "Uber retive Formveränderungen des Kernkörperchens," 'Archiv. für Mikrosk. Anat.,' bd. ix.

[5] L. Auerbach, 'Organologsche Studien,' heft i, pp. 167 and 168.

the disappearance of the vacuole which is seen in these at certain moments. I observed, also, that the very numerous nucleoli in the young ova of the frog likewise execute movements which consist in changes of form. I made these observations a little while after having established the alternate disappearance and reappearance of the nucleoli in the nucleus of the *Gregarina gigantea*.[1] Since then I have several times observed the same fact in the *Monocystis lumbricorum*. In the latter species, however, there exists one nucleolus of larger size, which is more voluminous than the rest, and which never disappears, but which changes its form. and in which one sees vacuoles appear and disappear: sometimes the nucleolus encloses only a single very extensive vacuole; a few seconds later it may show a crowd of little ones of all dimensions; at other times there are no longer so many. Other researches and numerous occupations have, however, hindered me from publishing these observations before now. I have not seen changes of form take place under my eyes in the ova of the Starfish; but I have no doubt whatever that the differences shown to exist in the form of the germinal spot must be attributed to the contractility of the substance of the nucleoli. This conclusion results from observations made on mature ova of changes which essentially consist in the reduction of the nucleolus into fragments, a reduction which immediately precedes the disappearance of Wagner's spot.

The pseudo-nucleoli, eight to fifteen in number, are corpuscles of very variable size, composed of a substance which is much less refractive than the nucleolar matter. Sometimes they are disseminated throughout the whole extent of the germinal vesicle; more frequently they are situated in the neighbourhood of the true nucleolus. They have an entirely different composition, and different properties from the latter. It is incorrect, therefore, to say with Hoffmann that there are from one to ten nucleoli in the ovum of the *Asteracanthion rubens*.

Hertwig, in his work, declares himself a partisan of the opinion of Auerbach, who holds that the membrane of the germinal vesicle, and of nuclei in general is produced by the differentiation of a thin layer of protoplasm around a vacuole which is filled with the nuclear substance. Although I do not wish here to go fully into my views on the subject of the

[1] Édouard van Beneden, "Sur une nouvelle espèce de Grégarine, Désignée sous le nom de Gregarina gigantea" (On a New Species of Gregarina, named the Gregarina gigantea), 'Bull. Acad. Roy. de Belg.,' 2nd series, vol. xxviii. See also translation of same in this Journal.

constitution of the cell-nucleus in general, and the germinal vesicle in particular, I think it right to say that I do not in the least coincide in this opinion of Auerbach and Hertwig. A young nucleus is formed by a homogeneous substance which I have called the nuclear essence.[1] When this young nucleus enlarges, the nuclear essence becomes united with a substance which is taken up from the protoplasm of the young cell. The nuclear substance which results from this union forms the body of the nucleus. The membrane of the nucleus, as well as the nucleoli, are the unmodified remains of the primitive young nucleus; they are formed exclusively by the nuclear essence. At the moment when a nucleus is about to divide the nucleoli as well as the nuclear membrane dissolve in the nuclear substance. Hence it results that the contour of the nucleus becomes scarcely distinguishable, and that the nucleoli disappear. All who have studied the multiplication of cells know how little the nucleus is apparent at the moment when the division of the cell is about to take place. It is this fact which has given rise to the theory according to which all cell-multiplication is preceded by the disappearance of the nuclei. The momentary disappearance and reappearance of the nucleoli in the nucleus of the Gregarina were mentioned by me in 1869. I was at that time unable to give any interpretation of these facts; still, they bear witness to the facility with which the nucleolar substance dissolves in the nuclear substance. The observations of which I shall give an account later on, and which show that the spot of Wagner dissolves in the germinal vesicle with the disappearance of the latter element, form an argument which may be turned to account in support of my opinion. Immediately after the dissolution of the nucleoli and of the membrane in the nuclear substance, a separation is effected between the nuclear essence which goes to form the equatorial zone and the nuclear liquid which is driven back to the poles of the nucleus. The latter, after the division of the zone into two nuclear discs which are to become new nuclei, loses itself in the body of the cell.

The vacuoles which appear in so large a number of the nucleoli are, I think, nothing but the result of the momentary union of certain parts of the nucleolar substance with the nuclear liquid.

I believe that this way of looking at the constitution of the nucleus is the only one which can explain the physical and

[1] Édouard van Beneden, "De la Maturation de l'œuf, de la Fécondation et des Premières Phénomènes du Developpement Embryonnaire des Mammifères," p. 50.

microchemical characters of the element and of its various parts—to wit, the nuclear membrane, the body of the nucleus, and the nucleoli. It depends entirely on the phenomena which one knows in relation to the vital manifestations, the development and the multiplication of nuclei.

If one breaks the membrane of the egg of a Starfish so as to allow the contents of the egg to escape into weak osmic acid or picric acid, the germinal vesicle presents a peculiarity which I have been able also to make out in the living egg, after having first observed it in the germinal vesicle isolated and treated with these reagents. In the nucleus of the egg there exists a network with large meshes, formed by a very finely granular substance. It is in this reticulum that the pseudo-nucleoli are found; the germinal spot appears to be the centre from which the reticulated filaments start. The characters of this network vary, moreover, in different ova; sometimes one even sees in place of the network a small granular collection formed by the substance of the reticulum and the pseudo-nucleoli. This reticulum I have also found in the rabbit, and I have proposed to designate the substance which constitutes it by the name of *nucleo-plasma*. The first to describe a network similar to that of the Starfish was W. Flemming.[1] He found that in the Anodons and the Unios the transparent liquid of the germinal vesicle is traversed by numerous anastomosing filaments. Kleinenberg has mentioned something similar in the germinal vesicle of the fresh-water Hydra; and lastly Hertwig has observed it in the *Toxopneustes lividus* and in the ova of the mouse. I do not know that anything of a similar nature has been shown to exist in other nuclei of cells. I think, therefore, it will not be without interest to mention here my observations on the constitution of the nucleus of an enormous cell which constitutes by itself alone the whole central part of the body (Leibeshöhle of Kölliker) of the Dicyema. The nucleus, which is more or less regularly ellipsoid in shape, presents a thick membrane, beneath which there exists a very close network composed of a finely granular material, whilst the contents of the nucleus (*nuclear substance*) are perfectly homogeneous and transparent. The body of the nucleus is traversed in certain individuals by a reticulum which makes it resemble a spongy tissue; in other individuals there exists only a bundle of filaments like pseudopodia. I have represented one of these nuclei (figs. 20 and 21). By the application of the picrocarminate after previous treat-

[1] W. Flemming: "Studien in der Entwickelungsgeschichte der Najaden," 'Sitzb. der K. Acad. der Wissensch. zu. Wien,' vol. lxxi.

ment with osmic acid, the nuclear substance is stained of a rose colour; the nucleolus and the membrane bright red; the reticular substance does not stain. This is also the case with the nucleo-plasmic meshwork of the germinal vesicle of the rabbit.

But if one opens the ovary of the starfish at the moment of sexual maturity one finds in it not only ova similar to those which I have just now described, and which are essentially distinguished by the fact that although they have attained the dimensions of the ripe ovum they still have their germinal vesicle in the centre of the vitellus; together with these ova one sees others which have not yet attained maturity; others in every respect similar to those of which I have before spoken, but differing from them in the fact that the germinal vesicle has become superficial; and others which show no longer any trace of the vesicle of Purkinje. These latter, however, are ova which are only exceptionally observed; generally speaking, all the ovarian ova still possess their germinal vesicle.

The ova in which the germinal vesicle has reached the surface of the yolk scarcely differ from those above described; they have an ellipsoidal or spheroidal form; their zona pellucida, swollen by the seawater, is very thick, and its surface quite irregular. The yolk invariably presents the same characters; the germinal vesicle has retained its spherical form, and all the sharp definition of its contour. It is difficult to make out whether it is in immediate contact with the zona pellucida, or whether it is separated from that membrane by a thin layer of vitelline protoplasm. Inside the vesicle are seen the nucleus and pseudo-nucleoli in the midst of a little cluster of granules. I have never found the nucleo-plasmic network in the germinal vesicle when it has become superficial, whatever the method to which I have had recourse to convince myself of its presence. From the time when the germinal vesicle has taken its peripheral and superficial position, the nucleo-plasma, together with the pseudo-nucleoli, forms a small nucleo-plasmic mass by the side of the nucleolus.

If the ovarian ova of a completely developed ovary are received into a small vesicle containing sea-water, and if a fragment of testicle which has reached maturity be shaken in it for a moment, a certain number of ova are fecundated, and two or two and a half hours after having performed the artificial fecundation, divided ova are found at the bottom of the vessel. If one has taken care to leave only a small number of ova in the vessel, and to renew the water from

time to time the embryonic development advances rapidly, and at the end of from two to three days the ciliated embryos swim freely in the water. Artificial fecundation was performed for the first time in an Echinoderm by K. E. Von Baer; after him several embryologists have had recourse to the same procedure in order to study the development of Echinida, Asterida, and Holothurida. I will mention only Derbès, Krohn, Busch, J. Müller, A. Agassiz, and Selenka.

If, some few seconds after having performed artificial fecundation, one places a certain number of ova on an object glass, having taken them with a pipette from the bottom of the vessel in which the sexual products have been mixed, one observes that a crowd of spermatozoa have collected together at the surface of the zona pellucida. They move their tails with such force, that they even succeed in making the ova move. If, in order to study the successive phenomena which take place in it, one chooses an ovum which presents a superficially situated germinal vesicle, and if one watches it continuously, one finds that three quarters of an hour or an hour after the fecundation, the germinal vesicle which was so distinct at the time of the commencement of the observation, has completely disappeared. Subsequently one sees the vitellus undergo in succession the phenomena of retraction; distinctive bodies and polar globules appear in the peri-vitelline fluid; then the primary vitelline globe breaks into two parts. The ova, therefore, which are provided with a superficial germinal vesicle, are fit and liable, as well as those which no longer showed any traces of it in the ovary, to be fecundated. In order to be sure whether the disappearance of the germinal vesicle is the consequence of fecundation, or whether it occurs independently of the action of the semen, it is sufficient to turn some ovarian eggs into another vessel, taking care to avoid all mixture with the spermatic fluid. If one observes under the conditions above described ova taken from the bottom of the vessel, one can follow and observe the successive phases of the disappearance of the vesicle, just as when one follows them in fecundated ova. This disappearance then is independent of the action of the spermatozoa. This conclusion might, moreover, be drawn from this fact, that some of the ova lose their germinal vesicle, whilst still in the ovary, and that nevertheless these ova are perfectly fertile.

It was of the highest importance to study the successive phases of the disappearance of the vesicle, in order that *we might* be able to determine with certainty *how* it

disappeared. Nothing is easier than to make this observation on the ova of the starfish, since the eggs continue to develop on the object-slide, and the changes which they undergo take place under the very eye of the observer. Eight or ten times have I seen the series of modifications which lead to the disappearance of the vesicle of Purkinje evolve themselves under my very eyes. The succession of these phenomena is the same, whether one observes a fecundated ovum or follows them in a non-fecundated one. The whole series of these changes is completed in the same time in either case. I believe, however, that fecundation is often the immediate cause of the disappearance of the germinal vesicle, in this sense, that in a ripe ovum the germinal vesicle disappears from the time that the ovum is placed in the presence of the sperm, whilst that element might have still remained for some time if the ovum had not been fecundated. This appears to me to be shown by the following observation: if we place in two glasses ova from the same ovary, and fecundate those in the one, carefully preventing the sperm from becoming mixed with the contents of the other, an hour after the fecundation all the mature ova of the first glass will have lost their vesicle, whilst most of those in the second still show it perfectly distinct. If amongst the fecundated ova one be chosen which shows a quite superficial germinal vesicle, we may be almost certain to see the germinal vesicle disappear in less than an hour. But it is not so if we select from amongst the non-fecundated ova one which presents similar conditions.

Let us now see the series of modifications that are observed.

1. At first the little granular mass which is situated by the side of the nucleolus, and which is composed of nucleoplasma and pseudo-nucleoli, becomes less and less apparent; soon it becomes impossible to distinguish it; the germinal vesicle now contains only an entirely homogeneous and transparent liquid; with no other granule than the spot of Wagner or nucleolus.

2. The contour of the germinal vesicle becomes paler; the same is the case with the nucleolus, the substance of which appears to become less and less refractile. At the same time the nucleolar vacuoles become united with a single central vacuole, which appears as a clear spot, circumscribed by an irregular ring formed of a highly refractile substance. The nucleolus becomes very irregular, its surface is now knobbed, and the projections are separated from each other by fissures. The nucleolus resembles a small raspberry-like mass.

3. The nucleolus (germinal spot) breaks up abruptly into a large number of fragments which continue to diverge from one another and to spread themselves out into the whole mass of the germinal vesicle. These fragments are of unequal size. There is one of them which is notably of larger size than all the others, and which contains the central vacuole of the old germinal spot. This vacuole is now only surrounded by a thin layer of nucleolar substance, which as looked at appears only as a narrow and irregular ring. The formerly homogeneous contents of the germinal vesicle are now granular, and hold in suspension small bodies of variable form and dimensions which are only the fragments of nucleoli.

4. All the nucleolar fragments increase a little in volume and become less and less refractive. Soon they appear as only little clouds with ill-defined contours, forming spots on the uniformly homogeneous ground of the germinal vesicle. They at last completely disappear from view. The fragment of the nucleolus which encloses the central vacuole is still visible when all the others have already disappeared. Soon afterwards the last traces of this body likewise disappear. The germinal vesicle, which is still perfectly spherical, is now quite clear and transparent. We can no longer see in it any trace of nucleolus, nor any granule of whatever kind. The contour of the vesicle has become less and less marked, as if the substance of the membrane were dissolved at the same time with the nucleus in the nuclear substance. The progressive diminution of refractiveness of the nucleolar substance proceeds side by side with the vanishing of the contour of the germinal vesicle.

5. Some seconds after the last traces of Wagner's spot have disappeared, the membrane of the germinal vesicle becomes torn, or rather a hole is formed in it. This solution of continuity always appears in that part of the vesicle which is turned towards the centre of the ovum. The contents of the vesicle immediately flow out through the hole, forming a clear drop outside the vesicle. This little drop has the appearance of a bud or of a hernia. It enlarges very rapidly. At the same time the membrane of the vesicle withers and becomes wrinkled. The germinal vesicle has now withdrawn itself slightly from the surface and has got nearer the centre of the vitellus. It is enveloped on every side by the vitelline protoplasm. At one moment it appears as if formed of two clear masses adjacent to each other, which on account of their homogeneous appearance encroach upon the granular ground of the vitellus. One of these is formed by that part of

the nuclear substance which is still enclosed in the wrinkled membrane of the germinal vesicle, the other is formed by a drop of nuclear fluid expressed and projecting into the vitellus.

6. The extravasated drop is flattened against the vesicle, in the neighbourhood of the hole by which it made its exit. The nuclear mass then reassumes a more or less rounded form. But in this clear mass one distinguishes an irregular line, which separates the intravesicular from the extravesicular portion of the nuclear substance.

This line is produced by the membrane which separates the two parts of the nuclear substance which has become very thin. This line at last completely disappears, proving that the membrane is entirely disolved in the nuclear substance. There then no longer remains any portion of the germinal vesicle, except a clear spot, whose ill-defined contours become more and more irregular. The spot becomes smaller and smaller, and ends by disappearing completely. It seems as if the clear and homogeneous matter of the germinal vesicle became granular from the periphery to the centre. This appearance is probably the result of the progressive dissolution of the nuclear subtance by the vitelline protoplasm.

The successive phenomena which precede the complete disappearance of the germinal vesicle are these: 1. The solution of the nucleoplasmic mass and of the pseudonucleoli in the nuclear juice; 2. The breaking up of the germinal spot into fragments, and the progressive solution of these fragments in the nuclear substance; 3. The perforation of the membrane followed by the partial expulsion of the contents of the nucleus; 4. The complete solution of the membrane in the juice of the germinal vesicle; 5, lastly, the solution of the nuclear substance in the vitelline protoplasm.

The modifications which I have shown to exist in the nucleolus, the reduction of the vacuoles into a single vesicle, the changes in the form of that element, and its breaking into fragments cannot be explained unless we admit the contractility of the nucleolar substance. This view is moreover in conformity with the conclusion which one has been able to draw from the amœboid movements which have been seen to be executed by the nucleoli of other cells.

The facts which I have just related have not been observed by M. Hertwig in his *Toxopneustes lividus*. M. Hertwig thinks, on the contrary, without however, being able to affirm it from direct observations, that in that

Echinoderm the germinal spot passes out of the germinal vesicle, and becomes free in the vitellus, and there forms the nucleus of the yolk. But if I may judge from the figures which he gives of the germinal vesicle in process of retrogressive metamorphosis, I am convinced that the spot of Wagner in that Echinoderm undergoes the same fragmentation which I have mentioned in the starfish. I believe that the germinal bodies which M. Hertwig figures in the germinal vesicle (figs. 3, 4, 5, and 6) are nothing but the fragments of the nucleolar substance. It may be remarked that in his work M. Hertwig gives no information on the subject of these granules: he does not describe the phenomena which relate to the progressive metamorphosis of the vesicle; he confines himself to saying: "At the time of maturity of the egg, the germinal vesicle undergoes retrograde metamorphosis, and is driven by contraction of the protoplasm to the surface of the yolk. Its membrane is dissolved, its contents liquefy, and are finally absorbed by the yolk; the germinal spot, however, appears to remain unchanged, to penetrate into the yolk-mass, and to become the permanent nucleus of the ripe fertilizable egg."[1]

Of all the observations published up to the present time relating to the history of the germinal vesicle, the only ones which present any analogy with those which I have made on the starfish are those of Kleinenberg, on the fresh-water Hydra.[2] Kleinenberg, in fact, recognised that in that animal the germinal spot of the mature ovum undergoes a retrogressive metamorphosis; it presents an irregular and angular contour; then it breaks up into little fragments, and these at last dissolve. So far as concerns the spot of Wagner, the description of Kleinenberg is as applicable to the Starfish as to the Hydra. As to the description which he gives of the mode of disappearance of the germinal vesicle, it differs very notably from what I have seen in the Starfish. But at the end of his description Kleinenberg says: "Once I thought I saw an actual hole in the membrane of the germinal vesicle. If this is a normal phenomenon, it would be possible for the contents of the vesicle to escape and mingle with the surrounding plasma." I believe that the formation of the hole which Kleinenberg believed he saw is a normal phenomenon, and that it is by the formation of this hole that the contents of the vesicle are partially eliminated, both in the Hydra and in the Starfish; and it is as a result of the rupture of the membrane that the

[1] O. Hertwig, *loc cit.*, pages 11 and 12.
[2] Kleinenberg, *Hydra*, page 24.

contents of the germinal vesicle became
vitelline protoplasm. This identity of the
lead to the disappearance of the germinal
fish on the one hand, and the hydra on the
nificant that it is needless to insist upon

The fact of the dissolution of the nucleolus
substance is not an isolated fact. I have
the alternate disappearance and reappearance
the nucleus of the Gregarinida.
fusion of the nucleoli in the body of the
precedes the division of the nucleus; and I
same fact in my researches on the
embryonic layers
of the nucleoli
substance wh
in the nuclear

When, eighteen
which I have just given
also of studying the origin of the
mode of formation of the first
was interrupted in my study of the
development by the opportunity
day when I had not any Asterida at my
tigating the origin of the sexual organs in
some organisms which came into my
From the time when I entered upon the
in the Hydractinia, I thought I saw the
at a positive solution of it. I abandoned
study of the development of the Starfish;
being able to resume it when I wished; but
I have not again had the opportunity of
searches. I have seen the directive bodies
formed under my eyes, and I am in a
fresh nucleus appears in the vitellus before the
tation; but I am not in a position to say
directive bodies appear, nor how the first
in the embryo.

If I compare the results of my study of the
vesicle of the starfish with my observations on the
I find a complete analogy as to the essential facts; but also
differences the importance of which I do not wish to diminish.

In the rabbit as in the starfish the germinal vesicle disappears in so far as it is a morphological element; no part formed by the germinal vesicle exists any longer in the ovum at the moment when the first embryonic nucleus is seen to appear; no genetic bond then exist between the germinal

vesicle or one of its parts and the first nucleus of the embryo.

But whilst in the Starfish the collection of the nucleoplasma, the germinal spot and the membrane of the germinal vesicle are dissolved in the nuclear fluid and secondarily in the protoplasm of the yolk—in the mammalia these elements are thrown off into the perivitelline liquid to form directive bodies, and only the contents of the germinal vesicle remain in the yolk. Since in the starfish directive bodies are eliminated by the yolk, it is probable that in the Echinodermata, as in Mammalia, these bodies are formed by the nucleoplastic substance on the one hand, and by the nucleolar matter, joined to the substance of the membrane, on the other hand. It must be admitted that this is a pure hypothesis. But, however this may be, it follows from my observations that in the starfish as well as in the rabbit, there is no filiation whatever between the germinal spot and the first embryonic nucleus.

II. There is a second point in the observations and views of M. Hertwig, which appears to me irreconcilable with the results of my researches on the rabbit.

Is the clear spot which appears in the cortical layer of the yolk protoplasm without granulations, and is the corpuscle which is found there, and which Hertwig regards as the head of a spermatozoon a cell-nucleus? Or is the clear spot a nuclear body and the corpuscle contained in it a nucleolar element having no morphological connection with a spermatozoon?

The *spermakern* of Hertwig is the head of a spermatozoon enclosed in a clear spot. This is composed of protoplasm without granulations. My peripheral pronucleus, which is certainly homologous with Hertwig's clear spot and with the peripheral nuclei of Auerbach, Bütschli, and Strasburger is, according to the terms employed in my preliminary communication, "a small, round homogeneous body, without granulations; it has, in fact, the appearance of a vacuole. But when treated by osmic acid, the clear substance of the so-called vacuole becomes darker and of a grey colour, while the substance of the yolk is coloured brown." It is only later when the pronucleus is already buried in the yolk, that several very highly refractive corpuscles, which would be taken for so many nucleoli, if seen in an ordinary nucleus, appear in its interior. If then our observations agree so far, that we have both seen the body which appears in the yolk, near the surface of the egg with (according to Hertwig) one, or (according to me) several refractive corpuscles, we differ *(a)* as regards the moment of appearance of the nucleoli-form

elements, (*b*) as regards the number of the elements. Moreover the interpretation of the observed facts is different. Hertwig regards the refractive corpuscle as a nucleus (*Spermakern*), and this nucleus is, according to him, only the head of a spermatozoon; the clear space in which it is seen is occupied by protoplasma belonging to the yolk. In my opinion the clear spot is the nucleus; the transparent corpuscles which afterwards appear are nucleolar elements, the mode of formation of which I have never been able to observe, but which, in my opinion, based upon the time of their appearance, their number, and even their characters cannot be heads of spermatozoa.

I will make in the first place two observations which are entirely in favour of M. Hertwig. (1.) M. Hertwig has seen the successive phases in the formation of the first cleavage-nucleus produced under his eyes, observing the same egg continue its development on the stage, while my conclusions rest on the comparison of a large number of eggs at different stages of development.

(2.) M. Hertwig's view, with respect to the significance of his spermatic nucleus, rests on a positive fact; he has seen a line start from this body and become continued beyond the yolk into a spermatic filament. For my own part I have not been able to account for the formation of my nucleolar elements, and my opinion as to their significance rests upon negative facts.

But all those who have made observations of the kind of which I am now speaking, will acknowledge how easy it is to deceive oneself about the meaning of a delicate line observed in the yolk of a large egg. It is easy to deceive oneself with respect to the continuity of such a line with a spermatic filament contained in a narrow space between the yolk and the ovular membrane. Hertwig, on the other hand, says himself that he has never seen a spermatozoon bury its head in the yolk, and this head become his spermatic nucleus. Hertwig's direct proof is then still wanting.

As to the opinion which Hertwig has expressed with respect to the nuclear nature of his spermatozoon-head, it appears to me that little can be said for it. I see no reason for calling the little body which he regards as the head of a spermatozoon, a cell-nucleus. The clear spot in which is contained the homogeneous corpuscle seems to me to present rather the character of a nuclear element. Still the name of *cell-nucleus* cannot be given either to the clear spot or to the corpuscle contained in it; for the element looking like a nucleus which is formed near the surface of the yolk does

not become the nucleus of the first cleavage-sphere till it has become united to another element having also the appearance of a cell-nucleus. It is for this reason that I have spoken of the peripheral body as the peripheral pronucleus, and of the element which is formed in the centre of the yolk as the central pronucleus.

If we grant what appears to me hardly to admit of doubt that the small highly refractive bodies of my peripheral pronucleus are homologous with the *spermakern* of Hertwig, and that in the same way as there are nuclei with a single nucleolus and nuclei with many nucleoli, so also there are pronuclei with a single corpuscle, and others with many corpuscles, then it seems to me that Hertwig's view of his spermatic nucleus being the head of a spermatozoon has little probability. In Mammalia the nucleolar elements of the peripheral pronucleus have certainly not this significance.

(1.) The pronucleus at the moment of its appearance is in the rabbit without any granulations. Now this would be inconceivable on Hertwig's hypothesis; the formation of the clear spot is, according to him the consequence of fecundation; now, fecundation begins with the presence of the spermatozoon. The formation of the spot must then always be consecutive to the penetration of the spermatozoon. The spot must accordingly be formed around the head of the spermatozoon, for the head can never penetrate into a preformed spot. We ought then never to see a peripheral pronucleus without at least one nucleolar corpuscle.

(2.) The pronucleus in Mammalia contains several granulations. These granulations have neither the appearance nor the dimensions of the heads of spermatozoa; they are spherical or oval globules, the dimensions of which show much variation; the smallest are almost points, and even the largest have not half the size of the heads of spermatozoa.

(3.) Auerbach, Bütschli, and Strasburger, observed before Hertwig and before myself, in the Nematodes, the Mollusca, and the Ascidians, sometimes one, sometimes several clear elements appear near the surface of the egg. All three describe these bodies as globules which are at first homogeneous, and are, at the moment they appear, quite without granulations. According to the observations of Auerbach, which on this point quite agree with all that I have observed in Mammalia, corpuscles, three to six in number, which Auerbach calls nucleoli, afterwards appear in the homogeneous liquid of these globules. There is not the slightest analogy between these corpuscles and the Nematode spermatozoa.

(4.) Refractive corpuscles, perfectly identical with those which arise in the peripheral pronucleus were seen also in the central pronucleus.

(5.) Corpuscles which perfectly resemble those observed in the peripheral pronucleus are found also in the completely developed nuclei of the cleavage-spheres, and these corpuscles are certainly nucleoli.

The opinion expressed by Hertwig that the transparent body which is formed near the surface of the egg is not a nuclear element, but protoplasm without granulations, appears to me untenable, so far as regards Mammalia, for the following reasons:

(1.) The transparent substance of the peripheral pronucleus does not behave with re-agents like the protoplasm of the yolk, but like the substance which forms the cleavage-spheres. If the pronucleus be treated with a one-per-cent. solution of osmic acid, it is not coloured brown like the protoplasm of the yolk; on the contrary, it appears on the brown ground of the yolk as a clear well defined spot. The pronucleus is faintly coloured pink by picrocarminate applied after osmic acid. By hæmatoxylin it is coloured violet-blue.

(2.) When the peripheral pronucleus has become attached to the central pronucleus, it presents after a time the same appearance as the latter, enlarges, and its outlines become more and more distinct. The nucleolar elements remain for a certain time; finally they disappear and the enlarged peripheral nucleus, not one of the refractive corpuscles, becomes the first embryonic nucleus.

(3.) My view is in harmony with the opinion expressed by Auerbach, Bütschli, and Strasburger, who have all through regarded the transparent bodies formed at the periphery of the egg as nuclear elements.

From all the foregoing considerations I conclude that Hertwig's view respecting the mode of formation, constitution, and morphological significance of the transparent body which appears near the surface of the egg in Toxopneustes lividus is very improbable. I regard this body, which is homologous with the peripheral pronucleus of the rabbit, as being a nuclear element, and I regard the corpuscle contained in it which Hertwig calls *spermatic nucleus* not as the head of a spermatozoon, but on the contrary, as a nucleolar element homologous with those which exist in large numbers in the peripheral pronucleus of Mammalia, Nematodes and Ascidians.

I have found the number of these nucleolar elements to be very variable in Mammalia. In the rabbit the number

varies from one egg to another. In all the eggs of Cheiroptera containing two pronuclei which I have examined, each of these elements contained a single nucleolar element.[1] These variations in the number of the nucleoli have also been pointed out by Auerbach in Nematodes, and by Strasburger in Ascidians.

A New Process *for* Examining *the* Structure *of the* Brain. *With a review of some points in the* Histology *of the* Cerebellum. By H. R. Octavius Sankey. (With Plate XIV.)

The methods usually adopted in the microscopical examination of the brain have all proved in my hands more or less unsatisfactory. I find that when thin sections of hardened brain are cut and stained, the dye does not sufficiently differentiate the various structures so as to render their form and arrangement obvious, while in teased preparations the shape of the cells, the connection of their processes, and the fibres of the brain are just as likely to be torn to pieces as to be separated from the substance which surrounds them.

The plan which I am about to describe will, I think, be found to overcome, to a certain degree, several of these defects. The dye which I employ causes the nuclei to appear black; the cells and their processes are rendered dark purple, while the rest of the section is of a faint purplish-blue colour, so that the processes and fibres are rendered by these means extremely distinct, and may often be readily traced to distances of a quarter or half an inch, and in some cases even to greater length.

For the sake of clearness of description I will divide my process into several stages:—

I. The first stage consists in making slices of brain, which should be made from the organ as it is obtained from the post-mortem room, neither hardened nor altered in any way by reagents. The sections should be cut as thin as practicable, but slices of one eighth of an inch in thickness will not be found too thick for the subsequent treatment. I find the following a convenient mode of making such sections. A large brush is to be fixed to the back of the left ring-finger by means of two elastic bands; the operator then holding a piece of brain in the left hand, slices it with a large knife kept constantly wetted with spirit by means of the brush.

[1] Edouard Van Beneden, 'De la Maturation de l'Œuf.,' &c., p. 18.

A large amputating knife answers well for this purpose. The sections should be cut in the direction from the operator. As they are made they should be wiped off the blade with the brush, and allowed to fall into a vessel containing about half a pint of water.

The fixing of the brush in the position described will be found to give very important assistance. The piece of brain, as freshly taken from the subject, is difficult to seize and to hold in one position while making the slices as described, and the attempt to hold the brush and the brain at the same time will be found to be a work of difficulty; and as it is desirable to take several sections from the same place, the piece must not be allowed to slip from the operator's grasp, as it would be very difficult to readjust it.

II. The next stage is to subject the sections to the action of the dye. The water in which the sections were placed is to be poured off, until there remains only just enough to cover them. To this there is to be added an equal quantity of a one per cent. solution of the dye, so that in fact the sections will be now in a solution containing half per cent. of the dyeing material.

The Dye.—The material that I have found most satisfactory in its effects is called aniline blue black. It can be obtained from Messrs. Hopkins and Williams, Cross Street, Hatton Garden. In the dry state this material is a blackish powder, not unlike gunpowder in appearance. It is very soluble in water, to which it imparts an intense purple colour. Its chemical composition is, I believe, not exactly known. I regard the use of this dye as an essential part of the process. I have experimented with some thirty or forty other dyes, but have had no results at all equal to those obtained by aniline black. It is also useful for sections made after the usual process of hardening, &c.

The sections should be allowed to remain in the dye for about twelve hours; twenty-four or even thirty-six hours' immersion does not, however, often injure them. The next step is to pour off the dye, and add clear water until all the colouring fluid has been washed away. The stained sections may be poured, with the water they are in, into a large shallow basin, and each slice floated into the middle of a glass slide, and removed carefully from the water and allowed to drain. As each section in this stage should be marked, I can recommend for the purpose a very convenient slide, invented by Mr. Hicks, and described by him in 'Quart. Journ. Microsc. Science.' In this slide the portion on which the label is usually gummed is of ground-glass, and will

receive a pencil-mark without danger of its being obliterated.

III. The next step in the process is to dry the stained slices upon the glasses. They should be placed in an airy and dry situation, but not, as a rule, subjected to heat, but to a current of air. In certain states of the atmosphere, however, they may require the assistance of a very moderate degree of artificial heat. Or should the sections be very thick, a slight amount of dry heat is required so as to anticipate any liability to putrefaction before desiccation has had time to occur. When the sections are dry it will be found that they are firmly adherent to the glass, and that they, as a rule, show no tendency to crack, except just at the edges. Should it be desirable to obtain a good view of the edge of any section, a slice of unstained brain may be placed over the stained piece so as to overlap the edge, which virtually removes the edge it is desired to see from its external position, and renders it far less liable to crack.

IV. When the sections are properly dried they will be found to be of the consistence of somewhat dry cheese, and rather uneven in thickness. The next step is to bring them to a condition suitable for microscopical examination, by reducing them to the necessary thickness. The sections, before they are reduced, may be described as consisting of three layers—an upper, middle, and lower, the latter being in contact with the glass. It is obvious that the upper and the lower, which were the parts in immediate contact with the dye, will be more deeply stained than the central layer, which in fact remains white. In reducing the thickness of the sections by paring, it is clear that if the upper layer is removed but one of the deeply stained layers will remain. The object to be gained by the paring is to reduce the section to the lower stained layer only. The unstained or intermediate layer may be removed as completely as practicable without endangering the deeper blue layer; but if any unstained substance be left it is of not much consequence, as the process of clearing renders it invisible. The operation of paring can readily be performed with a razor, but it can be much more efficiently and quickly accomplished by means of an instrument that I have devised for the purpose. This instrument is a kind of plane, and is a modification of an ordinary carpenter's two-inch skew rabbet-plane, but with which I operate in the reverse position, that is, with the cutting edge uppermost. The modification consists in screwing to the sides of the plane two slips of iron, having perfectly true and level edges, which are also adjustable by set

screws, and can be raised just above the level of the cutting blade of the plane. They thus forms guides, one on either side of the knife, by means of which the thickness of the section is regulated. In using the plane, each end of the glass side is pressed tightly down on the iron on either side, so that the slide bridges over the interval between the guides. The dried slice of brain being on its under surface, by passing the slide along the guides from front to back all the projecting portions of the tissue come in contact with the knife, and are pared off until the whole is reduced to the requisite thickness, this thickness being regulated at will by raising or depressing the side guides.

V. The preparation is now to be cleared by means of dammar varnish or Canada balsam. The intervention of oil of cloves is not required. The section may be examined at once with a low power, and, if found satisfactory, a cover glass should be placed over it.

Sections prepared as above may be made of almost any size. I have some which cover an extent of glass equal to three or four square inches. By employing a very long knife sections extending from the medulla oblongata through the pons varolii into the crus cerebri, for instance, can be readily prepared; and in many of my finished preparations fibres can be traced to extraordinary distances.

I have now treated the brains of a considerable number of animals by this method, and have also made a few preparations of the spinal cord and sympathetic and spinal ganglia of the larger domestic animals, and always with more or less satisfactory results. I find that the larger and older the animal is, the better are the preparations which are obtained. In the smaller animals the brain is softer and much more difficult to cut, and it is apt to break down in the dye into a ropy tenacious mass, showing no trace of the outline it previously presented. The brains of young and small animals become also more brittle when dried, and are liable to crack or contract in the process of drying. In spite of these difficulties, however, with care sections of even fœtal brains may be prepared. Of all brains, that of the human adult is the one for which my process is most suited, and especially, it has seemed to me, in cases in which the temperature has been elevated for some time previous to death. The process is inapplicable to brains which have been hardened by any reagent, as they will crack or become brittle in the drying; nor is it suitable for brains which have been in any fluid capable of crystallization; for crystals form during the desiccation and spoil the preparations. If sections cannot be made

as soon as the brain is removed from the skull, or at latest on the day following, the best fluid to place it in to preserve it is a strong solution of ammonium acetate of a sp. gr. of about 1040. This solution does not harden the brain to any marked degree, nor does it crystallize out during desiccation, nor by its deliquescent properties does it prevent the brain from drying. The brain, indeed, may be preserved in this solution for many weeks in a suitable state for the process. It should, however, be cut into pieces not larger than walnuts before being placed in the fluid, and should be soaked in water for several hours before the sections are made, otherwise it will be found that the brain has for some reason become much more adhesive, and sections when made cannot be removed from the knife without laceration.

Part II.

By means of the process of which I have now given the principal details, I have carefully examined many brains, with the view of testing various views which have been published at different times in connection with the Histology of the Brain and Nervous Centres. I propose here to give some of the results which my study has afforded me, and in the present communication I intend to confine my observations to the structure of the cerebellum; since my mode of investigation enables me to confirm some observations made originally by Dr. Obersteiner[1] concerning it, which, so far as I am aware, have not yet met with substantiation, and which do not even appear to be accepted, if I may judge from the fact, that though Dr. Meynert, writing in 'Stricker's Comparative and Human Histology,' alludes to several points mentioned in the paper quoted, yet omits all mention of those points to which I am about to allude.

Dr. Obersteiner, in speaking of the pure grey or outer layer of the cerebellum, says:—"The neuroglia of this layer is scattered over with round and elongated nuclei of a diameter of 0·007 mil. The latter scarcely present any cell around them, and probably belong to the connective tissue. A clear border surrounds the round nuclei, which is either round or angularly drawn out. It is these nerve-cells with processes which unite themselves to the end branches of Purkinje's cells." I find that if in a section made perpendicularly to the surface of the cerebellum, and at right angles to the lamellæ, one of Purkinje's cells be brought into view under a magnifying power of about 600 diameters and

[1] "Beiträge zur Kenntniss vom Bau der Kleinhirnrinde," 'Sitzungsbericht d. K. K. Acad. der Wissenschaft,' Band. lx, Heft i. Wien.

its peripheral process traced out, the following appearances will be observed. There is a slight variation in the detail, according as the cell is situated at the top or side of the lamellæ or at the bottom of the sulci, but the general arrangement is as follows:—(Fig. 1.) Each cell gives off in a direction toward the surface one primary trunk. This soon divides into two secondary trunks which pass to the right and left in a direction parallel to the surface, and in their progress send off at right angles numerous branches, which also are directed towards the surface or towards the pia mater; and, finally, the secondary branches, having gradually become smaller and smaller in their course, terminate themselves by turning, like the smaller processes, towards the surface.

At each point of division there is to be observed a small triangular swelling, which might aptly be compared to a small blot, such as might occur when a line of ink is drawn across another which is still wet. Various opinions have been broached as to the nature of this swelling. Dr. Meynert seems to have considered it due to a loose hyaline sheath which he describes as investing the cells, and as prolonged *a short distance on the larger processes*. Dr. Obersteiner, on the other hand, appears to think it due to the passage from one branch to another of fibres which do not pass back to the cells of Purkinje, but appear, as it were, to form a direct outer communication between the branches. I have been able to see distinctly this triangular enlargement at the union of some of the finest branches, such as are described below as connected with small cells in the pure grey layer, and which cannot, I think, be bundles of fibres, but must be single fibres. If such is the case, the enlargement cannot be produced in the mode supposed by Dr. Obersteiner. On the other hand, if this appearance is due to a sheath, then such hyaline investment must extend to the very finest fibres, and cannot cease at the point mentioned by Dr. Meynert.

The fibres running outwards may be seen to divide and subdivide into very fine branches, dividing, not three or four times only, as might be supposed from the drawings given in the text-books or by Dr. Obersteiner, but much more frequently. I have observed one branch to divide more than twenty-five times.

The more distant branches are given off at very a[cute] angles, and the fibres, after a very short distance, r[un] nearly or quite parallel course. The division is not st[rictly] dichotomous; for in many instances small branches aro[se]

off from the sides of larger ones, though more usually two branches of equal size result from the division of one. Dichotomous division prevails more as the fibres become smaller. The branches, having thus become finer and finer, are ultimately lost to view, or terminate in the remarkable manner described by Dr. Obersteiner (op. cit.).

In my preparations the union of the fibres with the protoplasm of the cells, in the pure grey layer, is plain and unmistakable, and resembles very closely the appearance described by Dr. Obersteiner, though some differences may be noted. I think it is probable that these are due to the mode of preparation of the object, and I believe that my method shows the structure to greater advantage than did the process at his command. Certainly I am able to see the structure, and the artist has been able to figure it much more decisively than Dr. Obersteiner has ventured to show it. (Fig. 2.) The fine fibres, on approaching the cells in which they are about to end, are seen in my preparation to enlarge and assume the character of the protoplasm of the cell, into which they pass without line of demarcation. Dr. Obersteiner, however, in his drawing, depicts the cell as a round ball, into which, in one sketch, a fine filament is seen to pass, and only into its proximity in his other sketch. Probably this appearance, as given by him, was due to the shrinking of the tissue in the process of hardening, for most of the cells seen in my preparations are not round, but, as Dr. Meynert describes them, triangular or pastille-shaped. Again, there is no clear space around the cell in my preparations, which in Dr. Obersteiner's drawing appears so wide as to be nearly equal to half of the diameter of the cell itself.

These small cells are not only seen to be provided with a process of union connecting them to the fibres in the manner described, but also to give off others, three or four in number, which are short, but which divide and subdivide until they are finally lost in the reticulo-molecular ground substance of the part. The "processes of union," which are connected with Purkinje's cells are usually directed toward that layer of cells, but this is not an invariable arrangement. My preparations corroborate Dr. Obersteiner's opinion that the terminal branches of the processes are directly united to cells, and that also side twigs are given off in a more or less rectangular direction, which also join cells, each fibre making for its cell a kind of stalk. I have, however, more frequently observed the former kind of connection than the latter.

With regard to the questions—are the processes invariably *connected* with cells? and are all the cells of similar character

and appearance connected with fibres? I can give no definite answer. I have observed that the cells in any given space that are unconnected with these processes are about equal in number to the terminal fibres, which appear to end indefinitely or appear unconnected with the cells, though there may be a slight excess in number on the side of the fibres. The more intensely the preparation is stained, the greater the number of those connections is there to be seen. I am therefore, inclined to believe that it is owing to the defective method of examination that we are still unable to assert that the fibres uniformly terminate in cells.

With regard to the statement of Dr. Obersteiner that there are nuclei of two kinds in the pure grey layer of Kölliker, I may state that I believe such to be the case. Firstly, there are those cells which are surrounded by a protoplasmic substance, and which are the most numerous; these are the cells which are connected with the fibres from the cells of Purkinje; and, secondly, there are undoubtedly others around which no protoplasm is to be seen; these are either round or more frequently slightly elongated, and both Dr. Obersteiner and Dr. Meynert regard them as free nuclei belonging to the neuroglia. In my preparation small arteries and veins may be detected, but no capillaries, or, if any, very few, and these but faintly indicated; but by taking a freshly stained portion of cerebellum and teasing it, the nuclei of the capillaries may be readily detected, and will be found to resemble in appearance, shape, and size the second kind of nuclei referred to above. I am therefore of opinion that this kind of nucleus belongs to the capillaries, and is not an element of the neuroglia. That such is the case, I think, derives support from the fact that their numbers are about what such an origin would readily account for.

The annexed chromo-lithographs represent with great accuracy the appearances seen in the preparations, both with regard to form and to colour. Fig 1 is magnified 140 diameters. It includes a part of every layer of the cortex of the human cerebellum.

Fig 2 is a highly magnified view (950 diam.) of a part of the pure grey layer of the cerebellum taken at a spot rather nearer to the pia mater than to the layer of Purkinje's cells. This figure is, to a certain degree, diagrammatic. The most central process, with its attached cell, which really occupied a position below and to the left of the structures represented, has been inserted in place of a fibre which did not show anything noteworthy. There was in the preparation, in the left upper corner, a small rent; this has not been represented,

group of six cells and nuclei taken from another part of the preparation having been inserted instead. Of the four nuclei in this group, three are elongated and appear to belong to the vessels; the most internal one, on the contrary, is round, and resembles those contained in the cells. Some clue to the existence of these free rounded nuclei may perhaps be afforded by the appearance seen at another part of the plate. In the right upper corner is a cell of which the protoplasm is so pale as scarcely to be visible, while nearer the middle of the lithograph is a nucleus nearly extruded from its cell. In the lower part of the plate are to be seen three or four unequivocal instances of union of fibres derived from Purkinje's cells with the protoplasm of small cells in the outermost layer of the adult cerebellar cortex. Such connections are, of course, faithfully copied from the preparation.

On the Development *of the* Ova *and* Structure *of the* Ovary *in* Man *and other* Mammalia. By James Foulis, M.D. Ed. (With Plates XVI, XVII, XVIII.)

Contents.—Introduction. The Ovary of the Kitten. The Human Ovary: (*a*) Nature of the germ epithelium; (*b*) The relation of the germ epithelium to the peritoneal epithelium; (*c*) The manner of inclusion of the primordial ova and germ epithelial corpuscles in the stroma of the ovary; (*d*) Development of the egg clusters; (*e*) The development of the membrana granulosa. General observations on the development of the membrana granulosa in adult ovaries. General conclusions.

In the month of August, 1872, Professor Turner suggested as a subject of investigation, the structure of the ovary and the development of the ova, more especially with reference to the recently published observations of Waldeyer. On the 21st December, 1874, Professsor Turner read before the Royal Society of Edinburgh a paper setting forth the result of my observations;[1] but in the month of April of the same year, in my graduation thesis for the degree of M.D.,[2] I had already demonstrated that the eggs and follicular epithelial cells have a different origin.

In the year 1870, W. Waldeyer published his observations on the development of the ovary and ova.[3] In this beautiful

[1] "On the Development of the Ova and Structure of the Ovary in Man and other Mammalia," 'Transactions of the Royal Society of Edinburgh,' vol. xxvii, 1875.

[2] 'Contributions to the Normal and Pathological Anatomy of the Ovary and Parovarium,' April, 1874.

[3] 'Eierstock und Ei,' Leipzig, 1870.

memoir we find an interesting history of the views of earlier observers on the origin of the ova and Graafian follicles. Waldeyer refers to the work of Valentin,[1] who, in 1838, first demonstrated the branched and tubular glandular structure of the ovary, and he makes special mention of the observations of Pflüger,[2] who first described the ova in mammals as arising out of the epithelium lining tubular glands in the ovary, and the formation of Graafian follicles from such tubular structures. Formerly the ova and follicles were considered to be derived from the cells of the stroma of the ovary. The observations of Pflüger led to the publication of numerous works on the origin and development of the ova, and the tubular formations were soon discovered in the ovary of the human subject, first by Spiegelberg. In Waldeyer's observations, however, we have the most recent addition to our knowledge of the development of the ova and the formation of the Graafian follicles, and the following quotations from his work will place before us, briefly, the conclusions he has drawn from them:

In summing up, Waldeyer thus remarks:

"As the chief result of my investigations, it must be stated that both the egg and the follicular epithelial cells are derived directly from the germ epithelium. There is a reciprocal growth of vascular connective tissue and germ epithelial cells, in consequence of which large and small masses of the latter become imbedded more and more in the stroma of the ovary. The imbedded cells present a variety. Some of them, by simple increase in size, grow into ova, viz. primordial ova, while others keep to their original size, and by numerous divisions, at least as it appears to me, produce still smaller cells, viz. the follicular epithelial cells. A genetical distinction between primordial ova and follicular epithelial cells has consequently no existence. The germ epithelium is the common source of both."

In describing the ovary of a newly born child, Waldeyer thus states, in reference to its tubular structure:

"One sees long branching formations in the form of tubes, anastomosing with each other, as Valentin first described, and lying separate from each other at considerable distances. They pass upwards opening with narrow mouths into the epithelium, and appear as direct tubular gland-like processes of it.

"At the time at which the tubes described by Pflüger

[1] "Ueber die Entwickelung der Follikel in dem Eierstock der Saügethiere," 'J. Müller's Archiv. für Anat. u. Physiol.,' 1838.

[2] 'Die Eierstöcke der Saügethiere und des Menschen,' Leipzig, 1863.

exist, that is, as far as I can find, from the ninth month till a short time after birth, they present the structure ascribed to them by Pflüger, with the exception already mentioned, that there is as little of membrana propria in them as there is in the primary follicles. In the tubes, and mostly in the middle of them, as Pflüger described, we meet with egg cells distinguished by their size and form, often immediately concatenated one behind the other. Whether in the tubes new egg cells are formed, I cannot decide; but I think it likely, because here, as well as on the surface epithelium, some epithelial cells may develop into egg cells. Division of the egg cells in the tubes Pflüger seems to have observed, but I have not seen it in fresh specimens.

"Follicles are formed from the tubes as well as from the egg compartments, directly through the growth of interstitial issue. At the lower end of the tube, as may be well explained from the want of a membrana propria, interstitial tissue grows into the tubes and incloses the individual egg cells along with a portion of the not fully developed epithelial cells which surround them, and in this way primary follicles are produced.

"The tubes of Valentin and Pflüger can lay claim only to a secondary importance, and are not essential for the egg and follicle formation; the greater part of the follicles have undoubtedly an earlier existence, long before these tubes are formed."

My observations have been made on the ovaries of calves, kittens, cats, puppies, rabbits, human fœtus, &c., and in the present paper I have described what I have seen in the ovaries of young kittens and of the human fœtus with the object in particular of demonstrating that, whereas the eggs are derived from the germ epithelium, the nutrient cells of the ovum, or the follicular epithelial cells, are derived from the cells of the stroma of the ovary.

The Ovary of the Kitten.

I shall first describe the structure of the ovary and the development of the ova in a kitten of two or three weeks; and I may here remark, that I know of no animal better suited than the kitten to show the relation of the germ epithelium to the stroma of the ovary.

In a thin vertical transverse section of a two weeks' old kitten's ovary we may distinguish

1st, The germ epithelium.
2nd, The zone of egg clusters.
3rd, The fibrovascular stroma.

The germ epithelium consists of distinct copuscles arranged in a layer which passes round the ovary from one lateral border to the other, and becomes continuous with the peritoneal epithelium which covers the stalk or peduncle.

The corpuscles of the germ epithelium consist of clearly defined nuclei, all of which have a thin investing film of protoplasm. In some instances this protoplasm is very clearly made out, and is in considerable quantity, but in other cases it can scarcely be seen, even under very high powers of the microscope. As in the ovary of the fœtal calf, there is a constant proliferation of the germ corpuscles by a process of fission. They are somewhat granular, and vary considerably in size ; some are oval, but the greater number are spherical. In the ovary of a kitten of four weeks the corpuscles of the germ epithelium appear columnar in form and compressed laterally. In the round or spherical corpuscles, which are generally larger than the others, the nucleus is extremely well marked, and frequently possesses a bright nucleolus The spherical form of the larger corpuscles appears to be produced by the swelling out of the nucleus. In the two weeks' old kitten the corpuscles of the ovarian germ epithelium are several deep, and the layer itself is of irregular thickness.

As we examine the layer of germ epithelium as it passes round the ovary, we are at once struck by the fact that the corpuscles present a great variation in size and degree of development. Here and there we see large spherical nuclei, having round them a thin investing layer of protoplasm, while in other situations certain individual corpuscles stand forth prominently among their neighbours, and are conspicuous by their size and the size of their nuclei. In these latter the protoplasm surrounding the nuclei is in the form of a thick layer. Between these largest corpuscles and the ordinary small ones every variety of size and form is to be met with. The largest corpuscles, which present so much protoplasm round the nucleus, are evidently individuals which have reached an advanced stage of development. These have been termed primordial ova, and there can be no doubt that a great number of the larger or spherical germ epithelial corpuscles are developing into similar bodies.

The ordinary size germ epithelial corpuscles measure about $\frac{1}{2500}$th of an inch, while the primordial ova measure about $\frac{1}{1000}$th of an inch, but these measurements vary.

On carefully examining the primordial ova, in many instances two or more small fusiform corpuscles are seen in close contact with their yelk or protoplasm.

These fusiform bodies are about the size of the smallest germ epithelial corpuscles, and appear like connective tissue corpuscles, and in more than one case I have traced them in direct continuity with delicate bundles of minute fusiform bodies, which will presently be described as passing up from the deeper parts of the ovary towards the germ epithelium. In other cases, however, it appears to me that the bodies which lie in contact with the protoplasm of the primordial ova are germ epithelial corpuscles which have been displaced, pushed aside, or flattened out during the growth of the primordial ova. No cell wall can be made out round these primordial ova, but in certain sections one frequently sees a thin irregular stratum of hyaline substance passing round the young ovum in contact with its protoplasm, apparently growing up from the young connective tissue subjacent to the germ epithelium. In this hyaline substance two or three oval-shaped nuclei are seen. In some preparations the primordial ovum has tumbled out of its hyaline girdle, which remains like a ring standing up among the germ epithelial corpuscles.

In favorable specimens, in a single section of a three to four weeks' old kitten's ovary, I have counted as many as from thirty to forty large primordial ova among the ordinary germ epithelium corpuscles on the surface of the ovary. They may be recognised at once, for they appear like giants among their neighbours. Each consists of a large spherical nucleus, surrounded with a considerable layer of protoplasm; it is this latter substance which gives magnitude to the young ovum, as may be seen by the fact that though many of the nuclei of the larger germ epithelial corpuscles are nearly as large as the nuclei of the primordial ova, yet, being without the extensive investment of protoplasm, they do not appear markedly conspicuous among the rest like the primordial ova. Occasionally, two large spherical nuclei or germinal vesicles are found in a single large primordial ovum among the germ epithelial corpuscles on the surface of the ovary.

Among the germ epithelial corpuscles in the deeper parts of the germ epithelial layer all round the ovary we meet with many large primordial ova, and in contact with their yelk or protoplasm are small fusiform bodies, each consisting of an oval-shaped nucleus, around which is an investment of protoplasm drawn out at either end in a fusiform manner. Besides these primordial ova, numerous germ epithelial corpuscles, in various stages of development into the same, *are found* in this situation.

Immediately subjacent to the germ epithelial layer is the zone of egg clusters. In osmic-acid preparations this can be seen with the naked eye as a well-marked thick layer. The egg clusters are large oval-shaped and spherical collections of round corpuscles. The oval-shaped clusters lie close together, with their long axes directed from the centre of the ovary in a radiating manner towards the germ epithelium. Between the clusters, and sparating them, are delicate bundles or strings of small fusiform corpuscles, with blood-vessels, which may be traced growing upwards from the deeper parts of the ovary towards the germ epithelium. By far the greater number of the egg clusters are oval, but spherical-shaped groups or clusters are also met with, generally deeper in the ovary. Each egg cluster consists of a collection of corpuscles, most of which are spherical, and resemble very closely the larger corpuscles of the germ epithelium. If we direct our attention to any one egg cluster, it will be seen that the corpuscles vary in size, just as we described the corpuscles of the germ epithelium. At the lower part of each egg cluster, that is, farthest away from the germ epithelium, we find many large primordial ova, similar to those already described among the corpuscles on the surface of the ovary, and besides these are numerous large spherical nuclei, having round them protoplasm in layers of varying thickness. In each egg cluster it is possible to trace the corpuscles in all stages of development into primordial ova. Every large corpuscle in each egg cluster is potentially a primordial ovum. In contact with each primordial ovum in the egg clusters are small fusiform corpuscles, which may be traced as offshoots from the bundles of similar corpuscles which lie between and separate the egg clusters from each other. It is easy to compare the corpuscles in each egg cluster with the corpuscles of the germ epithelium, and to follow the steps of their development into primordial ova. In such a young kitten's ovary many of the egg clusters are still in connection superiorly with the corpuscles of the germ epithelium, but in most cases we find the bundles of young connective tissue which lie between the egg clusters have grown completely round them, separating them not only from each other, but also from the germ epithelium above. While the corpuscles of the germ epithelium are thus being enclosed in the meshes of young connective tissue, the nucleus of each swells out into a spherical body, around which is gradually produced that protoplasm which later constitutes the yelk of the primordial ovum.

When we trace the bundles of young connective tissue

downwards, we find they are offshoots of similar tissue of which the central part of the ovary consists. In the deeper parts of the ovary we find a very vascular young connective tissue forming the stroma of the organ, in which are imbedded numerous primordial ova, some in a far advanced stage of development. This stroma consists of minute fusiform corpuscles and blood-vessels. As the vascular bundles or strings of this tissue grow upwards between the egg clusters, delicate offshoots of the same insinuate themselves between the primordial ova and corpuscles in the clusters, and in this manner nourishment is brought within reach of these developing bodies. This interstitial growth begins at the lower part of each egg cluster, and gradually the primordial ova become separated from each other as the connective tissue thickens in between and around them, and they become at last included in separate meshes or primordial follicles. Where the egg clusters have not been completely shut in by bundles of connective tissue, the fusiform corpuscles of the latter may be distinctly followed up as far as the corpuscles of the germ epithelium, and, indeed, seem to disappear among them.

By the growth of the vascular tissue of the stroma among the imbedded corpuscles, the egg clusters in all parts of the ovary are gradually subdivided or broken up into single egg-containing meshes or follicles; and while this process is going on, the primordial ova are rapidly advancing in development. In the more superficial parts of the stroma subjacent to the egg clusters, and in the fibro-vascular zone of the ovary, the above-described process has already taken place. The primordial ova in some of the follicles are of very large size, and in the ovary of a four weeks' old kitten it is of great interest to compare the original germ epithelial corpuscles on the surface of the ovary with these large primordial ova now imbedded deep in the stroma, and we are thus able to observe what an extraordinary change has taken place in them during their development; and, what is of more importance, we recognise the nature of the change. Examined under a magnifying power of 1000 diameters, the ordinary germ epithelial corpuscle on the surface of the ovary appears as if it were of such a size that at least three of them would lie side by side on a threepenny piece without overlapping it; whereas the highly developed ovum imbedded deeply in the stroma appears as large as a florin or a half-crown piece, and the nucleus or germinal vesicle as large as a threepenny or fourpenny piece. Between *these two* extremes young ova in all stages of development

may be seen in the stroma of such a young ovary. The change which the germ epithelial corpuscle undergoes during its development is the following:

As soon as the corpuscle is imbedded in the stroma, its nucleus swells up into a round or spherical body, within which generally appears a spot or nucleolus. The nucleus presents a sharply defined limiting membranous wall, and becomes the germinal vesicle of the future ovum. Immediately around the nucleus is gradually produced that protoplasm which afterwards constitutes the yelk of the mature egg. *In all parts of the ovary, wherever we examine such young developing ova, we find fusiform corpuscles, like the fusiform corpuscles of which the stroma is composed, lying in contact with the protoplasm which surrounds the nucleus or germinal vesicle.*

The youngest egg clusters are immediately below the germ epithelium, and many of them are in connection with it. These latter have not as yet, been completely closed in and separated from the germ epithelial layer by the connective tissue bundles of the stroma.

The clusters of corpuscles in connection at their upper parts with the germ epithelium differ considerably from each other, both in size and form. Some are oval and elongated, others are spherical. Many of them are larger at their lower and middle parts than at the part in connection with the germ epithelial corpuscles. If a vertical section passes down through such clusters as these, such as are swollen out at their lower and middle parts but narrow at their upper parts, we have presented under the microscope the appearance as if bottle-shaped tubes full of round corpuscles extended from the germ epithelium downwards into the stroma of the ovary. These appearances are often seen, and it is important to study them well. It will be remembered that Pflüger[1] described in the young kitten's ovary numerous tubular processes passing downwards from the surface of the ovary into the stroma, in which the germinal vesicles originated, and by the successive divisions of which Graafian follicles containing eggs were formed. I have very carefully examined the ovaries of kittens and puppies, but have failed to find any tubular processes of epithelium passing into the stroma from which Graafian follicles are formed, and it appears to me that Pflüger is incorrect in stating that such exist in the ovary of the kitten.

In section, the ovary of a kitten at birth presents a

[1] Pflüger, 'Die Eierstöcke der Saügethiere und des Menschen,' Leipzig, 1863, p. 4.

structure very similar to that of the ovary of the two to three weeks' old kitchen, but we find in the germ epithelium layer very few primordial ova. Many large spherical nuclei, with a thin film of protoplasm round them, are seen among the germ epithelial corpuscles, and the germ epithelial layer itself is much thicker as a stratum all round the ovary. Most of the large egg clusters below it, at this stage of development, are in communication superiorly with the corpuscles of the germ epithelium, and separating these clusters delicate bundles of young connective tissue, made up of minute fusiform corpuscles, may be traced growing up between and around them. In the deeper part or fibro-vascular zone of such a young ovary the stroma is rich in blood-vessels, and numerous young eggs are imbedded in its meshes, but no large eggs, such as we described in the four weeks' old kitten, are found.

The Human Ovary.

In section, the ovary of a human fœtus of about seven months presents a somewhat triangular form. The ovary is attached to a stalk or peduncle consisting of fibro-vascular tissue, which passes into the ovary at the hilum and from the direct prolongations of which the whole stroma is derived. On examining a thin section of such an ovary under low powers of the microscope, direct prolongations from the stalk are seen proceeding in a radiating manner towards the periphery in all directions, and communciating with each other in such a manner that the whole stroma becomes arranged in the form of a mesh-work consisting of fibro-vascular tissue. In the meshes of this stroma are large and small collections of corpuscles. At the periphery of the organ the meshes are large, and the contained groups of corpuscles are correspondingly large; but as we pass deeper into the ovary the meshes with the included groups of corpuscles become smaller, till at last we find small meshes containing but one or two large corpuscles.

The surface of such an ovary is very irregular, presenting numerous small fossæ-like grooves and furrows, seen clearly under a low magnifying power.

Investing the ovary, and passing round it from one lateral border to the other, is a layer of columnar corpuscles. This layer is the germ epithelium, and as it invests the ovary it dips down into and lines certain tubular structures and tubiform depressions which, when seen in section, appear as passing down from the germ epithelial layer into the substance of the ovary.

(*a.*) *Nature of the Germ Epithelium.*—In a fœtus of seven and a half months the germ epithelium consists of columnar-shaped corpuscles placed side by side and arranged as an investment to the whole ovary.

The germ epithelial layer rests on a thin irregular stratum of connective tissue which is part of the general stroma of the ovary, and is formed by delicate processes of the same, which at an earlier stage of development grew upwards from the deeper parts of the ovary to surround and inclose in meshes those large groups of corpuscles found immediately under the germ epithelium in its entire extent. This young connective tissue stratum is the forerunner of the tunica albuginea.

On tearing to pieces in a little water a fragment of this ovary and examining the débris under high powers of the microscope, many small portions of the germ epithelium will be found. When the deep surface of the epithelial layer is examined, the corpuscles are seen to be placed close together, and present in the membrane a tessellated appearance. The chief part of each corpuscle is the nucleus. Around each nucleus is a small quantity of protoplasm which acts as a cement substance holding the nuclei together. When the germ epithelial membrane is looked down upon from above, immediately on bringing the corpuscles into view a clear space is seen round the nucleus of each corpuscle. This space is occupied by clear protoplasm. The corpuscles in this early stage of development appear as little nucleated pieces of protoplasm; the nucleus is always the conspicuous part of each corpuscle, but the protoplasm round it may vary in quantity. In each piece of epithelial membrane examined, the corpuscles are of different sizes. Some of the nuclei are swollen up into large spherical bodies, and around them is an increased quantity of clear protoplasm. In the larger nuclei a nucleolus is generally seen. The largest corpuscles are undoubtedly primordial ova. Between these and the smallest germ epithelial corpuscles every variety in size is met with.

When seen in profile, the germ epithelial corpuscles are columnar, but many of them are assuming an oval and spherical form. In the spherical ones the nucleus is clearly defined, and shows distinctly its well-marked membranous wall. Within the nucleus is a nucleolus, and around it is a thin film of protoplasm. In some instances this film is so fine as scarcely to be made out. When a section of a seven and a half months' fœtal ovary is examined under high powers of the microscope, in many situations among the

ordinary germ epithelial corpuscles all round the ovary, we find individuals standing forth prominently and conspicuous by their size and the size of their nuclei, similar to those bodies we described as conspicuous among the corpuscles of the germ epithelium in the kitten's ovary. Such have been termed primordial ova. On comparing these with the smaller round corpuscles, and these latter with the ordinary germ epithelial corpuscles, it is easy to see that they are germ epithelial corpuscles in a far advanced state of development. In the progress of growth the nucleus of the ordinary germ epithelial corpuscle first swells out and enlarges, becoming oval, then spherical, and around it is gradually produced that protoplasm which assumes such dimensions in the primordial ova. In contact with these primordial ova we often see small fusiform corpuscles, and as in the case of the kitten's ovary, some of them appear to have grown up among the germ epithelial corpuscles from the stratum of young connective tissue on which the germ epithelium rests.

It is a fact of great interest, that as the germ epithelial corpuscle becomes a primordial ovum, the nucleus which at first appears ill defined and semi-solid, and is surrounded by a comparatively small quantity of protoplasm, now shows a remarkably clear definition, and at last appears as a spherical vesicular body with a fine double-contoured wall, and within it one or two nucleoli are usually seen. The nucleus of each germ epithelial corpuscle becomes the germinal vesicle of the primordial ovum, and the nucleolus corresponds to the germinal spot.

The ordinary germ epithialial corpuscles measure in their longest diameter about $\frac{1}{1800}$th of an inch, and in their shortest $\frac{1}{3500}$th of an inch, but both these measurements vary considerably.

On referring to Waldeyer's work "Eierstock und Ei," plate ii, figs. 9, 11, 13, I find that he represents the germ epithelial corpuscles as little bodies in which the nuclei are comparatively small, while the protoplasm round these is in all cases very extensive. This is not in accordance with my observations. An examination of the ovaries of numerous fœtal and newly born animals clearly shows that in each ordinary germ epithelial corpuscle the nucleus constitutes by far the greater part of the corpuscle, the protoplasm around it being in the form of a fine film. In the primordial ova, however, the enlarged nucleus has around it a correspondingly large quantity of protoplasm, and then

these bodies present the appearance as described by Waldeyer.

(*b.*) *The Relation of the Germ Epithelium to the Peritoneal Epithelium.*—In a section of the ovary of a seven and a half months' fœtus, the stalk or peduncle is directly continued into the ovary to form the stroma, which we described as arranged in the form of a mesh-work. If we now direct our attention to the epithelium covering this stalk, we find it is directly continuous with the peritoneal epithelium, and on the other hand passes without a break into the epithelium which covers the ovary, but a gradual change in its character takes place as it slides into and becomes continuous with the latter.

In the peritoneal epithelium, as seen in profile, the nuclei of the cells are oval and flattened from above downwards, and are placed at a considerable distance from each other, and the protoplasm around them is extensive. Tracing this epithelium towards the ovary, as we approach the latter, we observe the nuclei of the epithelial cells to become round and columnar, and gradually to lie closer together until at last they are almost in contact. They then crowd together, and in the form of a thick layer pass on to the surface of the ovary as the corpuscles of the germ epithelium. As the corpuscles of the peritoneal epithelium gradually slide into those of the germ epithelium, we find the protoplasm which invests the nuclei of the former gradually becomes less and less in quantity, and the nuclei themselves become gradually larger and more columnar, until at last the nuclei pass on to the surface of the ovary as distinct columnar bodies, each having round it a fine investment of protoplasm. In thus tracing the peritoneal epithelial corpuscles into germ epithelial corpuscles we see the nuclei undergoing fission, and by this process crowds of germ corpuscles are produced. The whole germ epithelial layer must be regarded as a thick layer of proliferating corpuscles. From the first appearance of the ovary as an organ until its development is completed, there is a constant proliferation of the germ epithelial corpuscles; and in growth of the ovary, as fast as some corpuscles become imbedded in the stroma, others are produced to take their place.

It is interesting to recal to recollection the statement of Schenk[1], Waldeyer, and others, that in th first instance the whole peritoneal cavity is lined by a layer of columnar corpuscles, and when the Wolffian body appears in connection

[1] 'Beiträge zur Lehre von den Organ-Anlagen im motorischen Keimblatt.'

with Remak's middle plate, it likewise has an investment of similar corpuscles. The first appearance of the ovary is the thickening of this columnar epithelium on the median side of the Wolffian body (Waldeyer). At the commencement of this thickening there is seen under it a small out-growth of young connective tissue, continuous with and proceeding from the interstitial tissue of the Wolffian body. There can be little doubt that the thickening of the columnar epithelium is brought about by a proliferation of its corpuscles, and the proliferation itself is due to the influence on the corpuscles of the vascular young connective tissue subjacent to them. While the columnar corpuscles constantly proliferate to form the germ epithelial corpuscles from which all the future ova are derived, the rest of the peritoneal chamber becomes lined by a layer of flat epithelium, forming the peritoneal epithelium.

From the very first appearance of the germ epithelium of the ovary as a distinct structure, processes of the subjacent young connective tissue grow upwards among the corpuscles as the first step in that process of imbedding, whereby the corpuscles become surrounded in meshes of vascular stroma, and ultimately form ova. From its earliest condition till late in the development of the ovary, the germ epithelium cannot be stripped off as a layer from the ovary, because delicate processes of the ovarian stroma are constantly growing up between the corpuscles as fast as they are produced.

Pflüger states his opinion that the ovum is simply a peritoneal epithelial cell. With this statement I cannot altogether agree. Both the ova and the peritoneal cells are undoubtedly evolved from a common ancestral source—the columnar layer of the great peritoneal cavity; but whilst in one limited region the columnar corpuscles form the germ epithelium and become converted into ova, in the greater part of their distribution they become converted into the endothelium lining the peritoneal cavity. Hence the ovum is no more a modified peritoneal epithelial cell than is the peritoneal epithelial cell a modified ovum.

From the corpucles of the germ epithelium all the ova are derived. We recognise the germ epithelium by the fact that immediately below it, all round the ovary, are clusters or groups of corpuscles contained in meshes of the ovarian stroma, but under the peritoneal epithelium covering the stalk no such groups are found.

It is worthy of remark, however, that sometimes under those epithelial corpuscles, which, as it were, form a con-

necting link between peritoneal and germ epithelial corpuscles, we find small groups of round corpuscles apparently consisting of abortive ova. It is only under and in connection with the true germ epithelium that groups of true primordial ova are formed.

(*c.*) *The manner of Inclusion of the Primordial Ova and Germ Epithelial Corpuscles in the Stroma of the Ovary.*—In a section of the ovary of a human fœtus of three and a half months, one sees large strings of connective tissue corpuscles (fig. 1, *i*, *j*, *j*) growing upwards in a radiating manner from the deeper parts of the ovary towards the germ epithelium (*h*, *h*). These strings or bundles communicate with each other and form meshes, and in these meshes are round groups of corpuscles (*o*, *o*, *o*) which resemble very closely the corpuscles of the germ epithelium (*h*, *h*) which invests the ovary. Immediately under the germ epithelium such groups of corpuscles may be seen partially embedded in meshes of the stroma. In the deeper parts of the ovary we find large primordial ova (fig. 2, *m*, *m*) lying separate from each other, and in contact with the protoplasm which surrounds the germinal vesicle of each are small connective tissue corpuscles (*n*, *n*) exactly similar to those which make up the strings or bundles of young tissue in other parts of the ovary. These little connective tissue corpuscles are quite different in appearance from the corpuscles of the germ epithelium on the surface of this young ovary, or those imbedded in groups in the stroma.

The stroma of the human fœtal ovary of seven and a half months consists almost entirely of fusiform connective tissue corpuscles, and in a section of such an ovary we find the whole stroma arranged in the form of a mesh-work, in the meshes of which are large and small groups of corpuscles, just as we described in the case of the ovary of the calf, the kitten, and human fœtal ovary of three and a half months. In the bundles of connective tissue corpuscles which (as seen in section) lie between the groups of corpuscles we find blood-vessels, and on close examination it is seen that the walls of such blood-vessels consist of connective tissue corpuscles. Wherever the bundles of connective tissue proceed, in the midst of them are blood-vessels. On tracing the bundles of vascular tissue upwards, we find they arch round large and small groups of corpuscles immediately under the germ epithelium, and completely inclose them in meshes. In some situations under the epithelium the connective tissue bundles have not yet grown round the groups of corpuscles, but may be traced up as far as the germ epithelial corpuscles on either side of the

groups. Under the germ epithelial layer the youngest connective tissue is found, and in this situation it is the forerunner of the tunica albuginea. This youngest connective tissue appears as a transparent jelly-like substance, and in it are numerous fusiform corpuscles or nuclei. Fine homogeneous points of this young tissue may be seen insinuating themselves among the corpuscles of the germ epithelium, and in some preparations of six and seven and a half months' fœtal ovaries in which the germ epithelial layer is partially detached such fine points of the jelly-like young tissue may be seen in considerable numbers.

In many places immediately under the germ epithelium, small groups, consisting of a few germ epithelial corpuscles (fig. 3, *q*, *q*, *q*), are found in the act of being surrounded by this same jelly-like tissue; some of the groups are completely surrounded and separated from the germ epithelium layer, while others are partially surrounded, and are still in connection with the germ epithelium superiorly. The youngest connective tissue can always be traced in direct continuity with vascular bundles of tissue which completely surround the large groups of imbedded germ epithelial corpuscles, and is part of the general stroma of the ovary. This imbedding of germ epithelial corpuscles takes place under the germ epithelium all round the ovary.

After being thus included in meshes of the stroma, the germ epithelial corpuscles increase in number and in size, and there results the formation of those large egg clusters which are found under the germ epithelium in all parts of the ovary.

Each imbedded corpuscle undergoes the following change:—The nucleus enlarges, gradually becoming a spherical vesicular body, and the protoplasm which surrounds it is at the same time increased in quantity. As the result of the enlargement of each corpuscle in the group, the whole group as a cluster expands and becomes more or less spherical. As these egg clusters expand, those lying immediately under the germ epithelium push the latter structure before them, and in this manner the surface of the young ovary is made to present a very irregular appearance. Between the prominences or irregularities thus produced are depressions or furrows. These originate as simple depressions between two or more adjacent expanding egg clusters, and they become deepened by the growth and expansion of new egg clusters under the germ epithelium in connection with those already formed.

In describing the appearance of a seven and a half months'

human fœtal ovary in section, I stated that the germ epithelium passed round the ovary from one lateral border to the other, dipping into and lining certain tube-like structures and tubiform depressions which appear to pass from the surface downwards into the organ. Now, if vertical sections are made through a young ovary such as above described, whose surface presents numerous irregularities, we have the appearance presented as if tube-like structures (fig. 4, *p*,*p*) passed down from the surface of the ovary into the substance of the organ, and these are all lined by germ epithelium. Similar tube-like structures are produced when sections are made through the convoluted surface of a brain, and the grey matter might even be compared to the germ epithelium, inasmuch as it lines these sulci. But such structures are not tubes, nor are the similar appearances seen between the irregularities of the surface of a young fœtal ovary.

An examination of the young ovary, looking down on its surface from above, soon convinces us that there are no real tubular structures passing from the epithelium into the organ. On bringing the germ epithelium into view, and then slightly depressing the tube of the microscope, one sees large and small round groups of spherical corpuscles separated from each other by the connective tissue of the stroma, as was described by Waldeyer. Some of these groups are still in communication with the germ epithelial corpuscles, as may be seen in vertical sections where the knife has passed through a group of corpuscles not yet completely included in a mesh of the upward growing stroma.

In the germ epithelial layer which dips down into and lines the depressions of the surface of the ovary (fig. 4, *p*, *p*), we find the corpuscles undergoing changes during their development similar to the changes which the corpuscles of the germ epithelium in other situations undergo. At the bottom and sides of the sulci, among the corpuscles of the germ epithelium, we find large spherical nuclei with a thin investment of protoplasm, and large primordial ova, such as may be found in all parts of the germ epithelial layer, whether it lines depressions or passes over prominences of the surface of the ovary.

Frequently large egg clusters are formed under the germ epithelium which lines the furrows or sulci, and it often happens that the walls of these furrows come in contact; pushed together, as it were, by laterally situated expanding egg clusters. At the bottom of the furrows, where the epithelial walls come in contact, connective tissue passes through among the corpuscles, and in this way large egg

clusters become formed immediately below the germ epithelium at the bottom of the sulci. A vertical section passing down through such a sulcus and the group or corpuscles immediately below it, produces the appearance as if a tubular prolongation of germ epithelium was dilated at its lower part into a large sac, full of developing corpuscles of the germ epithelium.

During my investigations on the structure of the ovary and development of the ova, I have never found any real tubular structures, neither in the human ovary, nor in any other mammal that I have examined, such as the cat, dog, calf, sheep, guinea-pig, rabbit, and in no instance have I found Graafian follicles formed out of such structures in the manner described by Pflüger, Valentin, Spiegelberg, Waldeyer, and others.

(*d.*) *Development of the Egg Clusters.*—Each egg cluster is a group or collection of germ epithelial corpuscles enclosed in a mesh or capsule of the ovarian stroma.

The germ epithelial corpuscles on the surface of the ovary constantly produce new elements by the process of fission, and when included in a vascular mesh of stroma the corpuscles increase greatly in number by division, and from a few imbedded corpuscles a large group or cluster may be derived. It appears to me a most interesting and remarkable observation that these corpuscles, after a certain increase in number, expand or swell out into spherical bodies, and a careful examination of the egg clusters has convinced me that this change is brought about by the nucleus in each corpuscle swelling out into a spherical vesicular body, which afterwards becomes the germinal vesicle of the primordial ovum, and in close contact with the wall of the nucleus is gradully produced that protoplasm which afterwards forms the yelk of the ovum.

In each egg cluster we find certain individuals much farther advanced in development than the rest, and these appear exactly like the large primordial ova which we described as found among the corpuscles of the germ epithelium on the surface of the ovary. Each corpuscle in the cluster is potentially a primordial ovum. At the first there is but a small quantity of protoplasm round the nuclei of the corpuscles in each egg cluster, but as the nuclei enlarge and expand, the protoplasm round them is gradually produced in considerable quantity.

This development of the germ epithelial corpuscles into primordial ova takes place in the egg clusters in all parts of the ovary. All the imbedded germ epithelial corpuscles do

not reach the stage of primordial ova, many of them abort and disappear, and perhaps furnish a pabulum for the more vigorous and healthy ones.

In the ovary of a puppy at birth, one sees, in a beautiful manner, the egg clusters under the germ epithelium in all stages of development. Some of the clusters appear to consist entirely of large primordial ova, while in others we can trace the growth of the germ epithelial corpuscles into ova, and immediately under the germ epithelium are little groups of corpuscles in the act of being included in meshes of the stroma.

In following the further development of the ovary and ova, we notice that around each egg cluster is a well-marked capsule or mesh of vascular connective tissue. This connective tissue consists almost entirely of fusiform corpuscles and young blood-vessels. Just as at first, when delicate processes of the young stroma grew upwards among the ordinary germ epithelial corpuscles to include them in meshes, so now delicate processes of the young connective tissue with blood-vessels proceed from the walls of these meshes, and insinuate themselves in among the developing corpuscles in each egg cluster. As these processes thicken, the primordial ova gradually become separated from each other, and at last each is included in its own mesh or capsule of the stroma.

These single egg-containing meshes or capsules are the primordial follicles.

This formation of young Graafian follicles takes place in the egg clusters in all parts of the ovary, and at the same time new egg clusters are being formed under the germ epithelium in the manner described. In the ovary of a seven and a half months' human fœtus we find many newly formed egg clusters immediately under the germ epithelium, but below these, earlier formed egg clusters are in various stages of alteration into single egg-containing follicles, while deeper still we find a great number of young follicles all produced from the first-formed clusters in the way we have described (fig. 6).

In the first-formed Graafian follicles, which are situated most deeply in the stroma of the ovary, the young ova are of large size, and we are at once struck by the fact that the germinal vesicles in all are about the same size, although the protoplasm around them may vary considerably in quantity.

In each young Graafian follicle the ovum fits tightly; it occupies the whole cavity of the follicle; there is no space between it and the wall (fig. 6, *m*, *m*, *m*). The mesh of

stroma closely embraces the protoplasm of the ovum, and in almost every case we find fusiform connective tissue corpuscles (*n*, *n*) in the wall of the mesh lying in close contact with and indenting the yelk of the young ovum. Wherever we examine the primordial follicles, we see such fusiform corpuscles of the stroma lying in contact with and indenting the yelk of the contained ova. I called attention to the circumstance that among the germ epithelial corpuscles on the surface of the ovary primordial ova were found, having in contact with their protoplasm small fusiform corpuscles, which in some instances could be traced growing as offshoots from delicate bundles of similar bodies which formed part of the ovarian stroma.

(*e.*) *The Development of the Membrana Granulosa.*—The stroma of the human fœtal ovary is remarkable for the great number of connective tissue corpuscles it contains. Wherever we examine the stroma and its processes in all parts of the ovary, we find in it well-formed connective tissue corpuscles. In the middle parts of the ovary, where the stroma is well developed, the connective tissue corpuscles show very distinctly a central oval nucleus with nucleolus. Around the nucleus is a small quantity of protoplasm, drawn out at either end in a fusiform manner. Beside these we see naked nuclei and many small round bodies; in these latter the nucleus is in a state of division into two or more parts. These small round bodies appear to be swollen-out connective tissue corpuscles. In a well-formed connective tissue corpuscle the nucleus is comparable to the germinal vesicle of the ovum, and like it, at a certain stage of its development, it shows a sharply defined double contoured wall. In all parts of the ovary we find the connective tissue corpuscles dividing.

In various parts of the stroma we find delicate fibres developing into connective tissue corpuscles. The central part of such fibres becomes swollen out, and in this swollen out part a distinct oval nucleus appears; sometimes these fibres are direct prolongations of the protoplasm surrounding the nuclei of well-formed connective tissue corpuscles. The nucleus of a connective tissue corpuscle at first often appears as a solid or semisolid body, but it may become distinctly vesicular, like the nucleus or germinal vesicle of the ovum.

It will be observed that, in my description of the germ epithelium and of the development of the ova, I have avoided the use of the word cell, and substituted the term corpuscle, and my reason for not using the term cell in connection with the germ epithetial corpuscles is, that the germ

epithelial corpuscles, and the so-called columnar epithelial cells which line the pleuro-peritoneal cavity of the embryo, are nuclei which have a thin film or investment of protoplasm round them. These nuclei are the homologues of the nuclei of the peritoneal epithelial cells. When a corpuscle divides, each half of the nucleus carries with it a small investment of protoplasm. The protoplasm round the nuclei varies considerably in quantity during the development of the germ epithelial corpuscle. The term cell is employed in somewhat different significations by biologists. Some, for example, holding that a cell must have a definite wall, whilst others look upon the wall as of secondary and minor importance, and hold that a cell essentially consists of a nucleated mass of protoplasm. The germ epithelial corpuscles and connective tissue corpuscles do not possess a cell wall. When once a cell wall has formed round a corpuscle, the nucleus and cell contents may divide. The cell wall does not participate in the division, but incloses the products of the division. The cell wall may burst and liberate the contents of the cell.

Every egg cluster is included in a mesh of the stroma. This mesh consists of connective tissue corpuscles and minute blood-vessels whose walls consist of such corpuscles. Delicate processes of this vascular young tissue from the wall of the mesh grow inwards among the corpuscles which are developing into primordial ova. On tearing to pieces small fragments of a seven and a half months' fœtal ovary, and placing the débris in a little water under the microscope, we find small groups of primordial ova (figs. 7, 8), and single individuals (figs. 9, 10, 11), which have been torn away from the egg cluster, and in connection with some of the largest of these we frequently find fusiform corpuscles (figs. 10, 11, *n*, *n*) similar to those which lie in the walls of the meshes. In good specimens the fusiform corpuscles are found laying in indentations in the yelk substance (figs. 10, 11) which surrounds the germinal vesicle of the primordial ovum, and sometimes we see primordial ova whose yelk is indented in many places, but the fusiform corpuscles have been displaced from these indentations. No zona pellucida is found round such young primordial ova. The connective tissue corpuscles must therefore be in contact with the yelk of the primordial ova.

Directing our attention to the youngest follicles, we find these vary in size, but in every case the young ovum fills up the whole follicle in such a manner that its protoplasm presses against and distends the follicular wall. Little fusi-

form corpuscles in the walls, *and belonging to the stroma of the ovary*, indent the protoplasm of each young ovum as it lies in its follicle (fig. 6, *m*, *m*).

In an empty follicle from which the young ovum has been removed, these little connective tissue corpuscles appear as minute buds projecting into the cavity of the follicle from its wall.

In the deeper parts of the ovary numerous young egg-containing follicles are seen. In the youngest follicles but two or three fusiform corpuscles are found in contact with the yelk of the contained ovum. As the follicles increase in size and become older, the number of small corpuscles in contact with the contained ova also increases. In some we find seven or eight, and in still older follicles a perfect wreath of minute corpuscles is formed round the yelk of the young ovum. The oldest follicles are found in the deepest parts of the ovary, and in most of them there is a perfect wreath of minute corpuscles (fig. 12, *r*) lining the follicle. Now, from these young connective tissue corpuscles in the wall of the young follicles which lie in contact with and indent the yelk of the primordial ova, the corpuscles of the membrana granulosa are derived.

In describing the growth of fibre-like bodies in the stroma into connective tissue corpuscles, I stated that the middle parts of such fibres become swollen out, and in the swollen out part a distinct nucleus appears. This nucleus, though at first appearing semisolid, may become distinctly vesicular, and within it a nucleus is afterwards seen. By a careful examination of a whole series of young Graafian follicles, we trace the development of the corpuscles of the membrana granulosa in the following way:—Around the young ovum in each follicle the connective tissue corpuscles increase in number by division. As a single fusiform corpuscle divides, its nucleus appears to commence the division, and each half of the nucleus carries with it a small quantity of the protoplasm which originally invested the single nucleus. When a little wreath of such corpuscles is formed round the young ovum the nucleus of each copuscle swells out and beomes a distincly vesicular little body, presenting a very fine double contoured wall, around which is a small quantity of protoplasm. Within the nuleus generally a minute spot is seen. The protoplasm which surrounds the vesicular nuclei acts as a sort of cement substance, holding them together in the form of a capsular membrane round the young ovum. This capsular membrane is the first appearance of the membrana granulosa.

Only those connective tissue corpuscles which lie in actual contact with the yelk of the ovum in the follicle develop into corpuscles of the membrana granulosa; while those in the wall outside the membrana granulosa remain as connective tissue corpuscles, and follow the usual developmental process into ordinary fibrous tissue, of which the main part of the follicular wall at last consists. We shall afterwards see that in some of the oldest Graafian follicles in the adult ovary, in man and other mammals, which are about to burst to liberate the contained ovum, a great part of the wall of the follicle outside the membrana granulosa becomes again converted into large connective tissue corpuscles.

When first formed, the membrana granulosa consists of a single layer of minute corpuscles arranged in the form of a capsule round the ovum (fig. 12, *r*). As the young ovum enlarges, which it rapidly does after the formation of the membrana granulosa, it distends its follicle, and the corpuscles of the membrana granulosa increase greatly in number. In this membrane, when first formed, the corpuscles lie in close contact with each other, and when looked down upon from above they present a polygonal form from mutual compression (fig. 14, *r*). By the constant multiplication by division of its corpuscles, the membrana granulosa at last comes to consist of several layers. As a further stage in development, certain of the corpuscles, generally those in the middle parts of the membrana granulosa, break down, and become dissolved in a fluid which afterwards forms the liquor folliculi. By the breaking down and solution of these corpuscles, a cavity, the follicular space occupied by fluid, is formed. In section, this space appears semilunar in form. After the formation of this space the ovum is not entirely separated from the membrana granulosa, but remains connected with the wall of the follicle by a heap of corpuscles which surrounds it. In good specimens a layer of corpuscles remains in contact with the zona pellucida round the ovum for a long time after the formation of the follicular space. When first formed, the corpuscles of the membrana granulosa in the human fœtal ovary of seven and a half months measure about $\frac{1}{3000}$th inch.

For the complete demonstration of the development of the corpuscles of the membrana granulosa, the ovary of an adult rabbit is admirably suited. In a section of such an ovary, we first direct our attention to the structure of the stroma of the organ, and we find it consist entirly of very minute attenuated fusiform corpuscles. At first sight these appear as simple fibres, but each fibre is an elongated nucleus,

having round it a minute quantity of protoplasm. The stroma of the ovary of an adult cat has an exactly similar structure. In the adult rabbit's ovary, in a single section we may find young ova in various stages of development. In a very young ovum (fig. 15, *m*), we notice first the large germinal vesicle, with its germinal spot. Around the germinal vesicle is a small quantity of protoplasm, immediately in contact with which are several small fusiform corpuscles (*n*, *n*). These lie flattened against the ovum round its yelk, and are exactly similar to the fusiform corpuscles of which the stroma (*j*) is composed. Around an ovum slightly farther advanced in growth we find one or two of the fusiform corpuscles (*n*, *n*) have assumed a swollen condition, and in some instances individual corpuscles are in the act of dividing. In the case of an ovum still farther advanced in development, the corpuscles round the yelk substance have now assumed a spherical form, and as they increase in number and press against each other, they gradually become columnar (fig. 17, *r*).

As in the case of the human fœtal ovary of seven and a half months, the corpuscles of the membrana granulosa thus formed consist of vesicular nuclei placed close together, their walls being almost in contact, but a very minute quantity of cement material lying between them. When this young membrana granulosa is looked down upon from above, the corpuscles present a beautiful pavemented appearance. The nucleus of each corpuscle is polygonal from pressure by its neighbours (fig. 18, *r*). By the constant division of the corpuscles, the membrana granulosa soon consists of several layers (fig. 19, *r*), and a follicular space is formed by the breaking down and solution of some of the corpuscles. In the nearly ripe Graafian follicle of the rabbit's ovary one frequently finds several small follicular spaces in different parts of the thick membrana granulosa; and in a section made through such a follicle, bands or straps of membrana granulosa cells appear to pass from the ovum to the wall of the follicle. These bands were called "Retinacula" by Martin Barry. They are simply appearances produced when the section has passed through the walls or septa which separate several follicular spaces lying near each other.

In the ovary of the adult or old cat (fig. 21), we can trace in a beautiful manner the growth of the corpuscles of the membrana granulosa from the fusiform corpuscles of the stroma, which lie next to the protoplasm or yelk of the imbedded young eggs. In a section, we have first the epithelium on the surface. This consists of small flat polygonal

nucleated cells (fig. 22, h_1), about $\frac{1}{4000}$th or $\frac{1}{2500}$th part of an inch in diameter. This layer of epithelium is all that remains of the germ epithelium, and it can be stripped off from the ovary without difficulty. Below the epithelium, and passing round the ovary, is a stratum of connective tissue, consisting of elongated fusiform corpuscles. In the lower part of this stratum the fibres decussate freely. Immediately below this layer we come upon the remains of the large egg clusters which we described in the young kitten's ovary. A perfect zone, consisting of young eggs, lies immediately under the stratum of tissue which passes horizontally round the ovary, under the epithelium. In this zone the eggs lie very close together, many of them are in actual contact. In each egg we recognise the central germinal vesicle, with its spot or nucleolus—the germinal vesicles are all about the same size. Below this zone of eggs is the general stroma of the ovary, processes of which consisting of elongated fusiform corpuscles and fibres, grow in between and around all the eggs in the egg zone. These processes of stroma then become continuous with the horizontal zone of tissue which lies external to the egg zone, and with it grow round the ovary to form the tunica albuginea. As the processes of the stroma of the ovary grow in between and around the young eggs in the egg zone, fusiform connective tissue corpuscles (n, n) may be seen lying in contact with the yelk substance of the eggs, and these fusiform corpuscles are exactly similar in appearance to the corpuscles which make up the stroma (j, j) in other parts of the ovary. Around many of the young eggs the corpuscles of the membrana granulosa may be traced as they develop from these fusiform connective tissue corpuscles. The nuclei of these connective tissue corpuscles divide, swell up, and gradually form a wreath of little nuclei round the ovum. Each little vasicular nucleus has around it a small quantity of protoplasm, and in the nucleus a spot is generally found. By a constant division of these corpuscles the membrana granulosa at last consists of a thick layer, just as in the case of the Graafian follicle in the rabbit's ovary.

In the adult human ovary an exactly similar development of membrana granulosa corpuscles can be followed out. As the eggs lie imbedded in the stroma, the nuclei of those elongated fibres of the stroma which are in contact with the yelk substance swell up, and by constant division produce a wreath of little corpuscles round the ovum.

On comparing the epithelium on the surface of these adult ovaries with the fusiform corpuscles which lie round the young eggs imbedded in the stroma of the ovary, from which

the corpuscles of the membrana granulosa are produced, we at once see how altogether different they are in appearance, and how impossible it is that there can be any connection between them. The epithelium on the surface of the adult cat's ovary is separated from the deeply imbedded eggs by a thick layer of connective tissue, and while the corpuscles of the epithelium are flat, polygonal bodies, with oval nuclei, the corpuscles in contact with the imbedded young eggs are elongated fusiform bodies, similar to those which make up the stroma in all parts of the ovary. By carefully examining these fusiform bodies as they lie on the surface of the egg, and as they lie round the egg, as in a profile view, it is seen that they are entirely different from the epithelial corpuscles on the surface of the ovary, and they are parts of the ovarian stroma. These observations appear to me to prove very conclusively that Waldeyer's view as to the development of the cells of the membrana granulosa is untenable.

After a single layer of membrana granulosa corpuscles is produced round the ovum, the wall of the follicle outside this capsular layer becomes fibrous and vascular. The wall of a nearly ripe Graafian follicle is very vascular. In the rabbit's ovary, in a very young follicle, the corpuscles around the egg, which give rise to the membrana granulosa corpuscles, are at first minute fusiform bodies, and lie flattened against the ovum, when seen in profile. As they develop into the corpuscles of the membrana granulosa they swell up, and by pressure against each other become columnar. Now, immediately outside this layer of columnar corpuscles the tissue consists of minute fusiform corpuscles (figs. 17, 18, 19, *j*, *j*, *j*) and blood-vessels. In a nearly ripe Graafian follicle, just before bursting to liberate the ovum, these minute corpuscles in the wall of the follicle outside the membrana granulosa swell up and enlarge, producing large fusiform corpuscles (fig. 20, *j*, *j*) very similar to the corpuscles of the membrana granulosa at a certain stage of development. This condition of the wall of the ripe follicle is also well seen in the human ovary. In the wall of the nearly ripe Graafian follicle of the rabbit's ovary, we can trace these large fusiform corpuscles becoming more and more like the ordinary small corpuscles of the stroma, as we examine them in the more external parts of the follicular wall. I have ascertained by careful observation that, from the very first appearance of the Graafian follicles to their bursting, no blood-vessels pass into the cavity of the follicle to reach the ovum, and yet this body increases enormously in size in a short space of time. This great increase in size is brought about by the production of proto-

plasm or yelk round the germinal vesicle, even after the latter has reached a definite size.

The chief function of the membrana granulosa is to nourish the ovum during its development. From the first appearance of the ovum as an ordinary germ epithelial corpuscle, until the development of the embryo and its extrusion from the uterus, it exists as a parasite. On the surface of the ovary at first it is an ordinary germ epithelial corpuscle. In the ovary, during a certain stage of its development, it is simply surrounded by vascular nutritious tissue, of which, however, it forms no part, but is simply resting on it. Here it imbibes nourishment until it is thrown off from the ovary. It then passes into the uterus, where changes due to impregnation occur in it, and processes of its surface become imbedded in the vascular mucus membrane of the uterus, and by the agency of these nourishment is absorbed for the germ till it reaches a certain stage of development, when it is thrown off from the parent. In the uterus, as in the ovary, the ovum is simply surrounded by vascular nutritious tissue of which it forms no part, but simply rests on it, and absorbs nourishment from it for its own development.

In the old follicles, some of the cells of the membrana granulosa show a distinct but fine cell wall round the protoplasm which invests the nucleus. In some of my preparations of the rabbit's ovary, the division of the nuclei of the corpuscles in the membrana granulosa is excellently seen (fig. 13, *v*). In these older follicles the protoplasm round the nucleus of the membrana granulosa corpuscle is sometimes very extensive, forming a thick layer which may be found drawn out in a fusiform manner (fig. 20, *u*) at one or more points.

In certain parts of the adult cat's and rabbit's ovary, we find large patches of granular cells, and running in between the cells are processes of connective tissue. These large granular cells have nuclei, but they are indistinctly seen because of the granular nature of the substance which surrounds them. In most cases the nuclei appear as round or oval bodies with nucleoli. An examination of the patches shows that the granular cells are swollen up individuals of the membrana granulosa from old and probably ruptured follicles, and are undergoing a fatty degeneration, while at the same time connective tissue corpuscles from the wall of the follicle are growing in between them. In almost every old ovary we find large yellow patches, consisting of the cells we have now described.

At birth the stroma of the human ovary is well developed in every part, and is arranged in a perfect network throughout the whole organ. The ovary might be compared to a piece of sponge in whose meshes ova are contained. The ova in the human ovary at birth are very numerous. They may be fairly estimated at about 35,000 in each organ. In favorable specimens a section of such an ovary presents under the microscope a splendid object. In it the stroma appears to be actually saturated with young ova (fig. 5, *m*), in all of which we can recognise the germinal vesicle as a distinct spherical body, and sometimes two or more germinal spots are seen in it, and around each germinal vesicle is the yelk substance filling up the entire cavity of the Graafian follicle. In all parts of the stroma the minute connective-tissue-corpuscles crowd together, and in the deeper parts of the ovary, where the oldest Graafian follicles are situated, the connective tissue corpuscles in the walls of the follicles may be seen in contact with and indenting the yelk substance of the included young ova, and in these cases the development of the corpuscles of the membrana granulosa may be clearly followed out. In such a section, under the germ epithelium, the last-formed egg clusters have not as yet been completely subdivided by the connective tissure stroma into the ultimate egg-containing meshes or Graafian follicles, but this is rapidly approaching completion.

After birth, the inclusion of germ epithelial corpuscles by the ovarian stroma becomes less and less, until at the age of about two years the process has entirely ceased; for at this period of development the tunica albuginea has assumed a special character as a complete investment to the whole ovary under the germ epithelium. Most superficially its fibres are arranged in a stratified manner, and run horizontally round the organ; and in sections the general stroma of the ovary is seen in connection with the tunica albuginea in all parts.

From the earliest appearance of the ovary we have on the one hand a growth of germ epithelial corpuscles, and on the other a growth of vascular connective tissue. The germ epithelial corpuscles, in groups or clusters, become gradually surrounded by the vascular connective tissue, and as development proceeds, the connective tissue grows into the groups between the corpuscles, and these become at last separated from each other, and by the thickening of the tissue in, between, and around them, they are ultimately included in separate meshes of the stroma. At the same

time the imbedded corpuscles are developing into primordial ova. This intercorpuscular growth of connective tissue takes place in all parts of the ovary wherever groups of germ epithelial corpuscles become imbedded, and it continues until the inclusion of germ epithelial corpuscles from the surface of the ovary has ceased. It is in this way, and in this way only, that we have Graafian follicles formed. As already explained, the Graafian follicles are the ultimate meshes of the stroma, formed by the growth of the connective tissue around the developing primordial ova. As these young ova become surrounded by the vascular tissue, the connective tissue corpuscles in the wall of the follicles in contact with their yelk develop into the corpuscles of the membrana granulosa in the manner described. According to my observations, tubular structures have no existence in the ovary at any period of its development, and I have never detected the formation of Graafian follicles and the corpuscles of the membrana granulosa in any other manner than that I have indicated.

By the continued free growth of the vascular stroma throughout the whole organ, the walls of the Graafian follicles become greatly thickened. The oldest and largest Graafian follicles are situated in the deepest parts of the stroma; that is what we would naturally expect, for they were the first formed, and lie surrounded by a great number of blood-vessels which are branches of the large trunks entering the ovary at the hilum.

After birth the corpuscles of the germ epithelium gradually become smaller in size, losing their columnar form, till at the age of six years, in the case of the human ovary, they present the appearance of small flattened corpuscles (fig. 23, *h*). They are very small, measuring in their longest diameter not more than the $\frac{1}{3000}$th part of an inch. The epithelial membrane composed of such corpuscles can be stripped off the surface of the ovary without difficulty. In the human ovary at twelve years of age, the epithelium has preserved an almost identical appearance to that above described, the epithelial corpuscles still remaining of very small size. In the ovary of an adult, the epithelial corpuscles do not measure more than the $\frac{1}{3000}$th to the $\frac{1}{2500}$th part of an inch in diameter.

General Conclusions.

The following general conclusions have been arrived at in the course of my investigations:

The corpuscles of the germ epithelium are derived by direct

proliferation from those columnar corpuscles which invest the median side or surface of the Wolffian body, and which are continuous with the layer of columnar corpuscles that lines the pleuro-peritoneal cavity of the embryo in the early stages of development. The stroma of the ovary in the early stages of development is produced by a direct growth out from the interstitial tissue of the Wolffian body immediately beneath the germ epithelium on the median side of the Wolffian body.

The germ epithelial corpuscles proliferate by fission. In the human fœtal ovary of 7½ months they measure $\frac{1}{3500}$th to $\frac{1}{2000}$th of an inch in their longest diameter, and about $\frac{1}{3500}$th of an inch in their shortest diameter. Eath germ epithelial corpuscle is a nucleus surrounded by a thin film or investment of clear protoplasm. In the act of becoming primordial ova, the nucleus of each germ epithelial corpuscle swells up into a spherical body, within which is generally seen a nucleolus, and around which is produced clear homogeneous protoplasm which subsequently forms the yelk of the ovum. Germ epithelial corpuscles are seen on the surface of the ovary in all stages of development into primordial ova. In each primordial ovum the spherical germinal vesicle present a sharply defined limiting membranous wall. Within the germinal vesicle is the nucleolus or germinal spot. All the ova in the ovary are derived from germ epithelial corpuscles.

In all parts of the germ epithelium processes of vascular connective tissue stroma grow in between and around certain of the germ epithelial corpuscles, whereby the latter become more and more imbedded in the stroma of the ovary. Germ epithelial corpuscles are being constantly produced on the surface of the ovary, to take the place of those already imbedded in the stroma. The imbedded corpuscles increase in number by division, and the nucleus of each swells up into a spherical germinal vesicle, around which is gradually produced the yelk of the ovum. In all parts of the young ovary under the germ epithelium, groups of germ epithelial corpuscles become imbedded in meshes of the stroma. As each individual in the group swells up the nucleus or germinal vesicle becomes very distinct as a round or spherical body. From the swelling out of each germ epithelial corpuscle in the group, the whole group expands and becomes more or less spherical. Such groups of developing corpuscles are called egg clusters. Each egg cluster is enclosed in a mesh or capsule of vascular stroma of the ovary. Each imbedded germ epithelial corpuscle is potentially an ovum.

The stroma of the young ovary consists for the most part of fusiform connective tissue corpuscles, and blood-vessels. The walls of the young blood-vessels in the young stroma consist of connective tissue corpuscles. The connective tissue corpuscles are direct offshoots from the ovarian stroma, and are found in contact with the yelk or protoplasm of each primordial ovum situated among the germ epithelial corpuscles on the surface of the ovary. Wherever we find primordial ova we see connective tissue corpuscles in contact with the yelk of each. In all parts of the ovary we find the nuclei of connective tissue corpuscles dividing. Sometimes these corpuscles are swollen out into round bodies containing three to four nuclei. In each egg cluster several of the included germ epithelial corpuscles are in a much farther advanced stage of development than their fellows. From the walls of the meshes inclosing the egg clusters, delicate processes of vascular connective tissue grow in, between, and around individual corpuscles in the egg clusters, and by a continued intergrowth of the young stroma in this manner each individual of the group becomes at last enclosed in a separate mesh or capsule. These last formed meshes are the Graafian follicles.

As a rule, each Graafian follicle is occupied by one young ovum. The protoplasm or yelk of each ovum is in close contact with the wall of each Graafian follicle. In contact with the yelk of each young ovum, and indenting it, are connective tissue corpuscles, which form part of the wall of each Graafian follicle. In the formation of the membrana granulosa, these connective tissue corpuscles in the wall of the Graafian follicle, and in contact with the yelk of the contained ovum, increase in number by division, their nuclei swell out into little vesicles, and at last a perfect capsule of such corpuscles is produced round the ovum. This capsule is the membrana granulosa or follicular epithelium of the follicle. At first the membrana granulosa consists of a simple layer of corpuscles lining the follicle.[1] The individual corpuscles of the membrana granulosa in the human ovary measures about $\frac{1}{3000}$th inch. As the ovum becomes mature, the corpuscles of the membrana granulosa proliferate, and then many layers of small corpuscles are produced between the ovum and the follicular wall. The cells of the mem-

[1] The formation of the granulosa from a single layer of connective tissue cells which are in origin independent of the germ-epithelium is paralleled by the mode of development of the inner cellular membrane of the egg-capsule in Cephalopods, as described by Ray Lankester, 'Phil. Trans.,' 1875, "Contributions to the Developmental History of the Mollusca."

brana granulosa are thus derived from the corpuscles of the connective tissue stroma, and not, as Waldeyer states, from the germ epithelial corpuscles. The follicular space is formed by a breaking down and probable solution of certain of the corpuscles of the thickened follicular epithelium in the midde parts of the same. The discus proligerus consists of follicular epithelial corpuscles, which are in contact with the zona pellucida of the ovum. The zona pellucida or vitelline membrane is formed by a hardening of the outer part of the yelk or protoplasm of the ovum, and is not, as Reichert, Pflüger, and Waldeyer stated, a product of the follicular epithelium. At birth the human ovary contains not less than 35,000 ova, few of which reach maturity.

In the human ovary at birth the germinal vesicles measure $\frac{1}{1500}$th to $\frac{1}{1200}$th of an inch. Most of them are about the same size, and present a sharply-defined membranous wall. In some germinal vesicles two or three germinal spots are seen. The tunica albuginea is the thickened stroma growing round the ovary. At the age of two and a half years all formation of ova from the germ epithelium has ceased.

Graafian follicles are not formed from tubular structures in the manner described by Pflüger, Spiegelberg, and Waldeyer. The appearance of tubular structures passing into the stroma of the ovary are produced by sections through furrows and depressions between irregular prominences on the surface of the fœtal ovary. The irregularities of the surface of the fœtal ovary are produced at first by the expansion of egg clusters upwards under the germ epithelium. When the walls of furrows and depressions come in contact, egg clusters are formed by the imbedding of germ epithelial corpuscles in that situation, just as in other situations. Egg clusters are formed in connection with the germ epithelium lining the furrows and depressions. Among the germ epithelial corpuscles lining the furrows, &c., we find large primordial ova, and corpuscles in all stages of development into the same, just as in other situations among the ordinary germ epithelial corpuscles.

In the human fœtus, round and oval-shaped groups of germ epithelial corpuscles are found in connection with the germ epithelium all round the ovary. When vertical sections are made through these they present the appearance as if tubular structures filled with developing germ epithelial corpuscles passed from the germ epithelium downwards into the stroma of the ovary.

The development of the corpuscles of the membrana granulosa, from connective tissue corpuscles of the stroma,

can be well followed out in the ovaries of adult rabbits and cats.

At the age of six years the epithelium of the human ovary consists of very small flat hexagonal-shaped corpuscles, measuring $\frac{1}{3500}$th to $\frac{1}{2000}$th of an inch. The corpuscles are undergoing division. This layer can be stripped off without difficulty. At the age of twelve the epithelium has little difference in appearance from the above, the small size of the epithelial corpuscles being remarkable. The epithelium is beautifully seen in old cats, and must be regarded as homologous with the peritoneal epithelium. In old cats the epithelium on the surface of the ovary consists of very small distinct cells, measuring from $\frac{1}{1800}$th to $\frac{1}{2000}$th inch, with granular oval nuclei.

Edinburgh.

On the GENUS ASTRORHIZA *of* SANDAHL, LATELY DESCRIBED *as* HAECKELINA *by* DR. BESSELS.

By W. B. CARPENTER, M.D., C.B., F.R.S. (With Plate XIX.)

[In consequence of the publication by Dr. Bessels in the 'Jenaische Zeitschrift,' vol. ix, of a description of the animal and test of Astrorhiza as a new genus, the following extract from a paper "On the Rhizopodal Fauna of the Deep Sea," presented to the Royal Society June 17, 1869 (abstracted in 'Proceedings of the Royal Society,' vol. xviii, p. 59), has been forwarded to us for publication by Dr. Carpenter.—EDS.]

ALTHOUGH twelve years have elapsed since Dr. Sandahl first characterised the genus *Astrorhiza*,[1] on the basis of specimens discovered in muddy shallows on the Scandinavian coast, its existence in British seas had not (so far as I am aware) been recognised previously to the Lightning expedition. My own attention was first drawn to this type about four years ago by Professor Lovèn, who was good enough to send me some specimens of it, at the same time informing me that it was not at all uncommon, and might probably be found on our own shores if carefully looked for. These specimens fully bear out the description given of the type by Dr. Sandahl, who characterises the genus as follows:—"Corpus discoideum, orbiculatum, testa tectum stellata e materiis diversis composita, sive poris, margine in radios plures tubulosos excurrente." The species is thus defined:—

[1] Ofvers. af. K. Vet.-Akad. Forh. d. 14, Oktober, 1847.

Color testæ stellatæ obscurus, griseo-brunneus, maculis parvis flavo-brunneis sparsus, inæqualibus, irregularibus, paullum intentibus. Numerus denticulorum varians (10-15). Diameter testæ (sine dentic.) 5-6 mm. Longitudo denticulorum 1-2 mm.

The diameter of the central disk in the specimens sent me by Prof. Lovèn commonly reaches one fifth of an inch, and its radiating prolongations sometimes attain a nearly equal length. The test is essentially composed of sandy particles of very irregular size and form, large rounded fragments being occasionally interposed among the ordinary angular grains, and the external surface being smoothed over by a kind of plaster, which seems composed of particles of fine mud, whilst the internal surface is roughened by the projection of angular sand-grains into the cavity, as in *Saccamina* (loc. cit.). These materials appear to be held together by an *organic* cement, which imparts a certain degree of flexibility to the envelope. The walls of the radiating extensions are composed of small sand-grains embedded in agglutinated mud, and the tubes thus formed are quite flexible.

A few specimens were collected by Mr. H. Brady about two years ago from the Dogger Bank, at depths of from 12 to 20 fathoms, which seem referable to this genus, though differing from Dr. Sandahl's type-form in some important particulars. The lenticular disk attains in some specimens a diameter of three tenths of an inch, but it is destitute of radiating prolongations. The sand-grains, which (as in Dr. Sandahl's specimens) are of very irregular size and shape, seem to be more firmly united together; but it still appears probable, from the flexibility of the envelope they form, that the uniting material is an organic, not a mineral cement. The external surface (so far as can be judged from specimens preserved *dry*) was not smoothed over by the plaster, which concealed all but the largest sand-grains in Dr. Sandahl's specimens.

The Lightning dredgings at 170 fathoms (temp. 41¾°) in the "cold area," and at 530 fathoms (temp. 48°) in the part of the "warm area" most nearly approaching this, where the Globigerina-mud was mingled with a considerable quantity of the fine sand characteristic of its bottom, yielded a remarkable series of arenaceous tests, presenting exactly the composition and mode of aggregation of the tests of the *Lituolæ* found in the same dredgings (loc. cit.), but exhibiting singular diversities in form, some of the most remarkable of which are represented in Plate XIX, figs. 1—13. These

seem referable to two fundamental types, which may be designated respectively the *lenticular* and the *cervicorn*. In the former the test may be considered as deriving its shape from a spheroid (fig. 5), which, whilst becoming flattened, sends out irregular digitate prolongations (figs. 6, 8, 10, 11, 12), so as very much to resemble the different aspects of an *Amœba*. In the latter (figs. 1 and 2) there is nothing that can be called a *centrum*, but a somewhat flattened stem puts forth irregular branches of the like shape, which often again branch, sometimes widening out into palmate expansions before doing so. Between these there are various intermediate gradations, the simpler or more elementary examples of the "cervicorn" type (figs. 4, 7, 13) having an obvious tendency to approach those modifications of the "lenticular" in which the radiations enlarge at the expense of the central disk. When the cavity is laid open, which is best done in the manner recommended in the case of the *Lituolæ* having the like texture (loc. cit.), it is found to be small in proportion to the size of the test, the walls of which are extremely thick (figs. 3 and 4). It is in all cases simple and undivided, and is occupied by a sarcode-body of a greenish-brown hue.

A very remarkable feature in this organism, which in other respects might be likened to a *Rhabdammina* (loc. cit.) of gigantic size and coarse construction, is *the absence of any definite aperture*. This I have carefully substantiated by the examination of a considerable number of specimens under various methods of observation; the extremities of the branches or radiations, like every part of the general surface, exhibiting a continuity of the arenaceous layer, save where it has obviously been interrupted by fracture. From the looseness with which the sand-grains are aggregated, however, it seems quite conceivable that the pseudopodial extensions of the sarcode-body may find their way out between them at *any* points, but more especially at the extremities of the branches or radiations, where the wall is thinner than it is elsewhere. And this deficiency of firmness in the test further suggests that it may undergo enlargement by the extension of its ramifications, the terminal layer of sand-grains being pushed away (so to speak) by the growth of the sarcode-body.

Any doubt I might have entertained as to the essential identity of our own types with the *Astrorhiza* of Sandahl have been removed by the fact that specimens collected by M. Sars, jun., from a depth of 450 fathoms, whilst corresponding in every particular with our own gatherings, are

unhesitatingly referred to that genus by Prof. Sars, who has kindly transmitted to me examples of them. The variety in the composition of the test would thus seem referable to the nature of the material furnished by the bottom on which these Rhizopods live, and partly to the condition of that bottom as regards stillness. We have seen that there are some arenaceous Foraminifera whose very symmetrical tests seem only capable of being built up where materials can be found of a precisely suitable kind, whilst there are others which appear able to use for their ruder constructions any material which the bottom may happen to supply; and *Astrorhiza* obviously belongs to the latter category. But the most curious difference between the original specimens and those more recently collected by M. Sars and ourselves lies in the manner in which these materials are united. For whilst in the latter the sand-grains are so slightly connected together by any intermediate material that the test is very easily disintegrated, the wall in the former has at the same time a considerable amount both of tenacity and flexibility—in this last particular differing from all other arenaceous tests with which I am acquainted. Now the extreme brittleness of the *Astrorhizæ* brought from great depths, where a profound stillness prevails, would be speedily destructive to them if they lived amidst the agitated waters of the surface; and it would seem as if the animal had the power, by exuding an organic cement, of so uniting the materials of its test as to give it just that power of resistance which is most suitable to its *habitat*.

NOTES AND MEMORANDA.

Relation between the Limit of the Powers of the Microscope and the Ultimate Molecules of Matter.[1]

The subject which I have selected for my address is the relation between the limit of the powers of the microscope, and the ultimate molecules of organic and inorganic matter. I think I may at all events claim for this question sufficient novelty. Until the last few years the subject could scarcely have been attempted, and even now so many necessary facts are imperfectly known, that nothing more can be done than to fill the gaps with plausible assumptions. This necessarily imparts more or less of a speculative character to some of my remarks; but it appears to me that in his annual address the president of a society cannot do better than endeavour to point out the general bearings of what is already known on some great question, even if for no other object than to prove the need of further inquiry.

Though fully impressed with the imperfect state of our knowledge, yet, even now, the facts are sufficiently definite to indicate, if not to prove, the existence of as wide a world of structure beyond the limit of the power of the microscope, as what has been revealed to us by it is beyond the powers of the unassisted eye.

I propose to divide my subject into three heads—

1. The limits of the power of the microscope.

2. The size of the ultimate molecules of organic and inorganic matter.

3. Conclusions to be drawn from the general facts.

In considering the limits of the power of the microscope, I shall assume that the instrument itself is perfect. and that the invisibility of the objects examined is in no way dependent on the want of the necessary characters. The point

[1] Abstract of the Anniversary Address of the President of the Royal Microscopical Society, H. C. Sorby, F.R.S., &c. With corrections communicated by the Author.

to which I particularly wish to direct attention is the limit of visibility depending on the constitution of light, beyond which light itself is of too coarse a nature to allow of our seeing objects distinctly defined. This question has been treated of in an admirable manner by Helmholtz in the jubilee volume of 'Poggendorff's Annalen' (1874, p. 573). The conclusion to which he arrives is that the limit depends on the confusion in the image due to the bright interference fringes overlapping the dark outlines of the object. This limit varies directly as the wave-length of the light, and inversely as the sine of half the angle of the aperture of the object-glass when illuminated by means of a condenser of equal aperture. According to this principle the limit for dry object glasses of 60° aperture is, roughly speaking, about equal to the wave-length of the light, and for the largest possible aperture equal to $\frac{1}{2}$ the wave-length. In the case of immersion object glasses of the same aperture, the limit is about $\frac{3}{4}$ of that for dry.

On comparing this theory with the results of observation, the agreement is very striking. It indicates exactly the same law for the increased defining powers of lenses of large aperture, as has been determined by experiment, and gives for the theoretical limit of distinct visibility $\frac{1}{30000}$th part of an inch, which is exactly the same as the mean of the experimental determination of a number of the most skilful microscopists. It also shows why in the case of lines at equal intervals, like Nobert's bands or the markings on Diatomaceæ, it is possible so to manage the illumination that the dark fringes of interference may coincide with the true lines of structure, and give rise to good definition, even beyond the normal limit, and also agrees with the fact that lines less than $\frac{1}{100000}$th of an inch apart can be *photographed*, though *seen* with extreme difficulty, if indeed truly resolved, except under very peculiar and exceptional conditions; since the waves of light at the blue end of the spectrum, which are concerned in photography, are short enough to give good definition of lines so near together that the interference fringes due to the longer waves at the red end would give an indistinct image. Taking everything into consideration, the agreement between observation and the theory is so close as to make it extremely probable, and to justify the conclusion that the normal limit of distinct visibility with the most perfect microscope is $\frac{1}{2}$ of the wave-length of the light. If so, we must conclude that, even with the very best lenses, except under special conditions, light itself is of too coarse a nature to enable us to define objects less than $\frac{1}{200000}$th or

$\frac{1}{100000}$th of an inch apart, according as a dry or an immersion lens is used.

The limit of $\frac{1}{80000}$ of an inch deduced on Helmholtz's principle from the physical characters of light agrees admirably with the estimate formed independently by various great authorities on the microscope. The mean of the estimate thus formed by Quekett, Moss, De la Rue, and Carpenter, as quoted by Stodder, is in fact exactly the same ($\frac{1}{80000}$ of an inch), so that we cannot, I think, be far from the truth, if we take that as the base on which to build further conclusions. With an immersion object-glass of very large aperture it might be possible to define an interval of somewhat less than $\frac{1}{100000}$ of an inch, but probably the above-named determinations were made with dry lenses. At all events, since the limit of visibility as determined by the use of the best modern microscopes agrees so completely with what appears to be the limit due to the physical constitution of light, we must, I think, conclude that our instruments do now enable us to see intervals so small in relation to the wave-length of light, that we can scarcely hope for improvement *as far as the mere visibility of minute objects is concerned*, whatever may remain to be done to improve their performances in other respects.

2. *The Size of the Ultimate Atoms of Matter.*

Having then come to the conclusion that the limit of distinct and unequivocal definition is somewhere about from $\frac{1}{80000}$ to $\frac{1}{100000}$ of an inch, it appears to me very desirable to consider what relation such a magnitude bears to the size of the ultimate atoms of organic and inorganic matter. From the very nature of the case the microscope altogether fails to threw any light on this question, and the only course as yet open to us is to draw the best conclusions we can from the various properties of gases. This problem has been attacked by Stoney,[1] Thomson,[2] and Clerk-Maxwell,[3] who, from various data, and by various methods of reasoning, have endeavoured to determine the number of ultimate atoms in a given volume of any permanent and perfect gas. In order to avoid inconveniently long rows of figures, I have reduced all their results to the number of ultimate atoms contained in a space of $\frac{1}{10^{10}}$ of an inch cube, that is to say, in $\frac{1}{10000000000}$

[1] 'Philosophical Magazine,' 1868, vol. xxxvi, p. 132.
[2] 'Nature,' March 31, 1870, vol. i, p. 551.
[3] Ibid., August 11, 1873, vol. viii, p. 298.

of a cubic inch, at 0° C. and a pressure of one atmosphere These numbers are as follows:

Stoney	1,901,000,000,000
Thomson	98,320,000,000,000
Clerk-Maxwell	311,000,000,000
Mean	50,260,000,000,000

As will be seen, there is a very great discrepancy between the numbers given by Thomson and Clerk-Maxwell. This is in part due to the fact that Thomson gives the greatest probable number, whilst Clerk-Maxwell has endeavoured to express the true number indicated by the phenomena of inter-diffusion of gases. The determinations do to a great extent depend on the measurements of length, and any differences are of course greatly increased when the number of atoms in a given volume is calculated, since that varies as the cube of the linear dimensions. Extracting the cube root of each of the above numbers, we obtain the number of atoms that would lie end to end in the space of $\frac{1}{1000}$ of an inch in length. It also appears desirable to give double weight to the determination by Clerk-Maxwell, since it is founded on the best data. We thus obtain as follows:

Stoney	12,390	29,275
Thomson	46,160	
Clerk-Maxwell		6,770
Mean		18,022

Calculating from this mean, we may perhaps conclude that the number of atoms in a cubic $\frac{1}{10000}$th of an inch is about 6,000,000,000,000. As will be apparent from the wide difference in the determinations, this result can be looked upon in no other light than a very rough approximation; but still, when we bear in mind that Thomson's result is given as a limit, it must be admitted that the numbers belong sufficiently to one general order of magnitude to justify our looking upon the mean as a tolerably satisfactory ground on which to form some provisional conclusions.

Now, if the gas containing the above-named number of atoms consisted of two volumes of hydrogen to one volume of oxygen, when combined to form vapour of water there would be a condensation of volume from three to two, and on condensing into a liquid a further contraction to $\frac{1}{1611}$ of the bulk of the vapour. Each molecule of water would however consist of three atoms of gas, and hence in order to determine the number of molecules of liquid water in $\frac{1}{1000}$ of an inch cube, it is necessary to multiply the number in a gas by $\frac{2}{3} \times 1234 \times \frac{1}{2} = 617$. This gives for the number of molecules

of water in $\frac{1}{1000}$ inch cube about 3,700,000,000,000,000. In this and all other cases I give round numbers, since any nearer approximation is impossible.

Though living organisms contain much water, yet far more complex substances enter into their composition. As an example of one of these, we may take albumen. According to Lieberkühn its composition is expressed by the formula $C_{72}H_{112}N_{18}SO_{22}$. It therefore contains seventy-one times as many ultimate atoms as water, and its atomic weight is about eighty-two times that of water. In the condition of horn I find that its specific gravity is about 1·31. Calculating from these data, I conclude that when the various constituents combine they contract to $\frac{9}{10}$ of the total volume, and not, as water, to $\frac{2}{3}$; and that the volume of a single molecule of albumen is about 55·6 that of a molecule of liquid water. If their form be similar, their diameter must therefore be 3·82 times that of a molecule of water. This would lead us to conclude that in a cube of $\frac{1}{1000}$ of an inch of horn there are about 65,000,000,000,000 molecules of albumen.

According, then, to these principles there would be in the length of $\frac{1}{8000}$ of an inch of about 2000 molecules of water, or 500 of albumen, and hence, in order to see the ultimate constitution of organic bodies, it would be necessary to use a magnifying power of from 500 to 2000 times greater than those we now possess. These, however, for the reasons already given, would be of no use unless the waves of light were some $\frac{1}{8000}$ part of the length they are, and our eyes and instruments correspondingly perfect. It will thus be seen that, even with our highest and best powers, we are about as far from seeing the ultimate constitution of organic matter as the naked eye is from seeing the smallest objects which they now reveal to us. Nor does there appear to be much hope that we ever shall see the ultimate constituents, since light itself is manifestly of too coarse a nature, even if it were possible to still further develop our optical resources. As matters now stand we are about as far from a knowledge of the ultimate structure of organic bodies as we should be of the contents of a newspaper seen with the naked eye at a distance of a third of a mile, under which circumstances the letters of various sizes would correspond to the smaller and larger ultimate molecules. This being the case, we may feel persuaded that particles of organic matter, like the spores of many living organisms scarcely visible with the highest magnifying powers, and, if seen, quite undistinguishable from one another, might yet differ in an almost infinite number of structural characters, just as any number of different news-

papers in various languages or with varying contents would look alike at the distance of a third of a mile.

3. *General Conclusions to be deduced from the above Facts.*

When we come to the application of these principles to the study of living matter, we are immediately led to feel how very little we know respecting some of the most important questions that could occupy our attention—questions which certainly never presented themselves to *me*, until I looked upon them from this point of view, and which perhaps have not occurred to any one before. As illustrations of the subject now under consideration I do not think I can select better than the facts bearing on the size and character of minute germs, and on Darwin's theory of ultimate organized gemmules, as described in Part ii, chapter xxvii, of his work on the variation of animals and plants under domestication. So far as I have been able to learn, he has nowhere given any opinion as to the probable *size* of such gemmules, nor discussed the probability of some of his speculations when examined from a *numerical* point of view, and in connection with the probable size of the *ultimate molecules* of organized matter. I therefore propose to do so; since, though not actually a microscopical question, it is most intimately connected with our studies, and as microscopists I think we have a good claim to investigate objects that are just beyond our magnifying powers.

For the sake of simplicity I will take into consideration only the albuminous constituents of animals, using the term albumen in a sort of generic sense, to include many compounds, which differ in many particulars, and yet have many in common. With slight modifications the same principles would apply in the case of other substances. Whatever be the special variety of this constituent, it is so associated with water in living tissues that in most, if not in all, cases they would cease to live if thoroughly dried. This is exemplified by the case of hair and horn, which must contain much water at the growing end, but are dead where hard and dry. In living tissues much of the water is no doubt present simply as a liquid mechanically mixed with the living particles, but it appears to me that we ought to look upon some portion as being in a state of *molecular combination*. So little attention has been directed to this kind of weak affinity, that its very existence is almost or quite ignored in many large and important chemical works, and yet probably many of the phenomena of life are mani-

fested only by such compounds. Very much light is thrown on this question by the study of the spectra and other optical characters of coloured substances. These clearly prove that when dissolved in any liquid the optical properties of the solution depend in part on the nature of the solvent, and are by no means the same as they would be if minute particles of the solid substance were diffused in the liquid. These facts cannot, I think, be explained unless we conclude that the solvent is to some extent in the state of molecular combination with the substance dissolved. This molecular affinity is also in some cases manifested by a swelling up of a solid substance when placed in some liquids, even when perfect solution occurs to a very limited extent. Such a condition appears to be very characteristic of the living tissues of animals, and makes it sufficiently probable that the ultimate living particles are molecular compounds with water, and not molecules of free dry albuminous substances.

Unfortunately, nothing definite is known respecting this question, and all that can now be done by way of illustration is to make some sort of a probable supposition. Taking everything into consideration, it appears to me that, as a reasonable example, we may assume that living albuminous tissue contains one-half of its volume of water mechanically mixed, and one-fourth its volume of free albumen united molecularly with an equal volume of water. On this supposition the number of molecules in $\frac{1}{1000}$ of an inch cube would be about

Albumen	17,000,000,000,000
Water in molecular combination	923,000,000,000,000
	940,000,000,000,000

Since, however, the form of minute living organisms more nearly approximates to spheres than to cubes, it will be more convenient to give the numbers in a sphere of $\frac{1}{1000}$ of an inch in diameter. For this there would be about as follows:

Albumen	10,000,000,000,000
Water in molecular combination	490,000,000,000,000
	500,000,000,000,000

In the present state of our knowledge it is perhaps impossible to say whether or not the essential characters of living particles are due to the structural arrangement of the molecules of this combined water as well as of those of the albumen, and whether or not in considering the possible variations in structure the total number of molecules should

be taken into account. The very small relative amount of dry matter in some living animals does, however, make it very probable that molecularly combined water really plays a part in their structure; and on the whole we may, I think, base our provisional calculations on the total number of molecules given above.

The Theory of Invisible Germs.

The relation between the size of the smallest object that can be seen, and that of the ultimate molecules of living matter, is manifestly a question of great importance in connection with the theory of germs. If the ultimate molecules were much larger than they appear to be, there would be serious objections to the theory; but, as far as we can judge, they are sufficiently small to make it possible for an almost endless variety of germs to exist, each having a distinct structural character, and yet each so small that there is no probability of our ever being able to see them, even as indefinite points. Thus, according to the principles described above, a sphere of organized matter one-tenth of the diameter of the smallest particle that could be clearly defined with our highest powers, might contain a million molecules of albumen and moleculary combined water. Variations in number, chemical character, and arrangement, would in such a case admit of an almost boundless variety of structural characters. The final velocity with which such extremely minute particles would subside in air must be so slow that they could penetrate into almost every place to which the atmosphere has access.

Darwin's Theory of Pangenesis.

Darwin's theory of pangenesis is an attempt to give something like a reasonable explanation of the phenomena of inheritance, and is not necessarily connected with the question of the evolution of new species. A full account of the theory will be found in his work on the variation of animals. At p. 374 of vol. ii. he says that "he assumes that cells before their conversion into completely passive or formed material, throw off minute granules or atoms, which circulate freely throughout the system, and when supplied with proper nutriment multiply by self-division, subsequently becoming developed into cells like those from which they were derived. These granules for the sake of distinctness may be called cell-gemmules, or, as the cellular theory is not fully established, simply gemmules. They are supposed to be transmitted from the parents to their offspring, and are generally

developed in the generation which immediately succeeds, but are often transmitted in a dormant state during many generations, and are then developed. Their development is supposed to depend on their union with other partially developed cells or gemmules which precede them in the regular course of growth."

He nowhere gives any opinion as to the actual size of gemmules, or the number present in particular cases, but it appears to me interesting to consider how far the theory will hold good when examined from this more physical point of view.

For the sake of argument, I assume that gemmules on an average contain one million structural molecules of albumen and molecularly combined water. Variations in number, composition, and arrangement would then admit of an almost infinite variety of characters. On this supposition it would require a thousand gemmules to be massed together into a sphere, in order to form a speck just distinctly visible with our highest and best magnifying powers. By calculation I find that a single mammalian spermatozoon might contain so many of such gemmules, that, if one were lost, destroyed, or fully developed in each second, they would not be completely exhausted until after the period of one month. Hence, since probably a number are concerned in producing perfect fertilisation, we can readily understand why the influence of the male parent may be very marked, even after having been, as regards particular characters, apparently dormant for many years.

In a similar manner I calculate that the germinal vesicle of a mammalian ovum might contain enough gemmules for one to be destroyed, lost, or fully developed in each second, and yet the entire number not be exhausted until after a period of seventeen years, and the entire ovum might contain enough to last at the same rate for no less than 5,600 years.

These calculations are made on the supposition that the entire mass is composed of gemmules. Of this there is little probability; but still, even if a considerable portion of the ovum consists of completely formed material and of mere nutritive matter, it might yet contain a sufficient number of gemmules to explain all the facts contemplated by the theory of pangenesis. The presence of any considerable amount of such passive matter in spermatozoa would, however, be a serious difficulty in the way of the theory, unless indeed very many spermatozoa are invariably concerned in producing fertilisation.

Taking everything into consideration, it does not appear

to me that any serious objection can be raised against pangenesis when examined from a purely physical point of view, as far as relates to the inheritance of a very complex variety of characters by the first generation, though there would have been many serious difficulties to contend with, if the ultimate atoms of matter had been very much larger than is indicated by the properties of gases.

When we come to apply similar reasonings to the second or following generations, we are compelled, along with Darwin, to conclude that gemmules have the power of producing other gemmules more or less closely resembling themselves, and of being collected together in the sexual elements, since otherwise the number that could be transmitted in a dormant state for several generations would be far too small to meet the requirements of the case.

Conclusion.

In my remarks I have made no endeavour to conceal our present ignorance of many very important questions connected with my subject. Want of the requisite data necessarily imparts a speculative character to many of my conclusions; but perhaps there is no more fruitful source of knowledge than to see and feel how little is accurately known, and how much remains to be learned.

PROCEEDINGS OF SOCIETIES.

Dublin Microscopical Club.

28*th October*, 1875.

Acetta coriacea from Oban Bay, new to the British Seas, in its simplest form.—Dr. A. Macalister presented a specimen of *Acetta coriacea* from Oban Bay, showing the simplest form of that species. The "personæ" were separate, each with a single "mouth." Hitherto the only recorded forms of this common sponge from the west coast were examples of the Aulophlegma-type, and Dr. Macalister believed this to be the first specimen of the simple form of this species recorded from the British seas.

Lejeunia microscopica, Taylor, *exhibited.*—Dr. D. Moore showed the rare and pretty *Lejeunia microscopica*, Taylor, which he had just brought from the west of Ireland; although to the naked eye little in the shape of an organized plant makes itself evident, this little gem, probably the very smallest of our higher Cryptogams, forms a very pretty object under a $\frac{1}{4}''$ objective.

Germinating Seedlings of Drosera.—Dr. Moore likewise drew attention to some germinating seedlings of Drosera; these had two ovate, smooth, cotyledonary leaves, the third leaf in all assuming the spathulate form of those of the species, and was clothed with the pretty characteristic insect-capturing glandular hairs.

Navicula Stokesiana, n.s., O'Meara.—Rev. E. O'Meara presented what he considered to be a new form of Navicula, of which the following is a description:—Valve large, rhombic-lanceolate, length ·0045″, breadth ·0018″; marginal striate band wide, inner striate band contiguous to the median line, narrow, and appearing to be elevated above the general surface, free space included within the inner margins of the inner striate bands narrow-linear, forming in the middle a very narrow stauriform line; space intermediate between the inner and outer striate bands occupied by lines of striæ which seem to be prolongations of the striæ of the outer striate band; striæ close, punctate, radiate. This form in most respects is similar to *Nav. irrorata*, Schmidt, 'Atlas der Diat.,' t. ii, f. 14; but it seems distinguished by the fact of having the intermediate space striate, whilst in *Nav. irrorata* this portion of the valve is represented as unstriate. Mr. O'Meara proposed under the name of *Navicula Stokesiana* to identify this beautiful and rare form with the name of the present respected President of the Royal Irish Academy.

Penium curtum, Bréb., *exhibited.*—On the part of Mr. Crowe

(who was absent) Mr. Archer showed examples of that somewhat remarkable desmid *Penium* (*Cosmarium*, Autt.) *curtum*, Bréb. These specimens were taken by Mr. Crowe from a shallow accidental water-deposit on the railway platform at the Bray station, thus showing the somewhat capricious distribution of this form, seemingly always occurring so far away from its congeners, the Desmidieæ at large, delighting, as they do, in boggy and mossy localities, not *roadside ruts!* Mr. Crowe had taken it on a former occasion on a roadside in Wales; again, scarcely a step from his own halldoor at Bray; and Mr. Archer on the roadside near Dargle Gate. This kind of habitat seems to accord with de Brébisson's description of that of *Cosmarium Regelianum*, (Näg.) Bréb., seemingly equivalent to *P. curtum*, Autt. (though de Brébisson records both as distinct); the former, at least, occurs, he says, "tapissant des cavités sablonneuses, recemment inondées par la pluie."

Sporocybe byssoides, Fries, *exhibited.* — Mr. Pim showed *Sporocybe byssoides*, Fries, which grew on a decaying bulb of *Sophronitis grandiflora;* the dense heads of brown, minutely echinulate spores were very characteristic.

Structure of petioles in the genus Nymphæa.—Mr. Mackintosh laid before the Club the results of some further examinations of the structure of the *petioles* in Nymphæa. Through the kindness of Dr. Moore, Director of the Glasnevin Botanic Gardens, he had obtained specimens of eight species, which he had found could be arranged in two groups. One of the groups, of which *Nymphæa alba* might be taken as the type, was characterised by having more than two primary air-passages of subequal size, and were provided with stellate cells. The other section, represented by *Nymphæa Devoniana* or *N. dentata*, had two large primary air-passages, several smaller secondary ones, and no stellate cells. Mr. Mackintosh exhibited sections of three species of the latter group—*N. lotus*, *N. Devoniana*, and *N. thermalis*—in order to draw attention to the only difference which he could detect in the different species: this consisted in the septum between the two primary air-passages being made up of two, three, or four cells. It might as yet seem doubtful if any dependence could be placed on a character of apparently so trivial a nature, but further investigation which he hoped to pursue would doubtless throw light on this interesting subject.—He likewise showed a cross section of the petiole of *N. lotus*, in which two or three of the cells bounding one of the secondary air-passages had become hypertrophied and grown out into a cellular mass which nearly filled up the cavity.

A Pseudo-Cosmarium—in other words, a Cosmarium-like exceptional state of an Arthrodesmus-incus-form—exhibited.—Mr. Archer drew attention to what at first blush looked like a new Cosmarium; and though this, as will be seen, was not so, he had actually labelled the bottle in which it occurred, in order to distinguish it from others, "Cosmarium inquirendum." This little form was very minute, of

quadratic general figure, but rounded at upper angles, and the constriction forming a rounded sinus at each side, the upper margin continuously with the "angles" broadly and gently convex, length and breadth about equal. It was thus different from any (British) Cosmarium. Never was a more deceptive little form, for it was not a species of Cosmarium at all, though, without doubt, desmidian. Some examination of the slide, indeed, soon showed that this quasi-Cosmarium owed its origin to an exceptional state of an *Arthrodesmus-incus*-form, that minute one, in fact, with simple cuneiform semicells and spines very slightly divergent. As is well known, when the growth of the new semicell, subsequent to division, is but partially advanced, the spines are not yet produced and the angles bluntly rounded; at this point, in several cases, the new semicell, as regards its own growth, had remained stationary, but, that notwithstanding, self-division had once more set in and new semicells were formed, the result of this second generation being these pseudo-Cosmaria. Here was a case, some might be found to advance, of the direct "jump" of one species into another "new" one! But it was not so. Cases were to be seen on the slide where some of these examples, which at the proper epoch had neglected to develop the spines, were now trying, as it were, to make up for lost time, and young short spines were at one end making their appearance. The whole thing was thus a mere "sport"—still a puzzling case of exceptional growth, such as Mr. Archer believed had not been before noticed, and therefore worthy of a passing record.

Euglypha spinosa, Carter, *exhibited*.—Mr. Archer, on the part of Mr. Crowe, who had taken the example in North Wales, exhibited *Euglypha spinosa*, Carter, a fine and large species, and most marked; it appears very rare indeed; as yet in Ireland it has only been found in Co. Kerry, and at "Toole's Rocks," Co. Wicklow, by Mr. Archer.

"*Headed*" *Bacterian exhibited and note thereon*.—Mr. Archer showed the active, and the stillglœogenous "Zoogloœa" condition of a bacterian seemingly very close to the "headed" form figured by Cohn in his memoir in his 'Beitr. z. Biol. d. Pflangzen,' Heft 2 (1872), t. iii, f. 13, though the actual identity seemed to be at least doubtful. Cohn's examples occurred in an infusion of dead flies; on the other hand, the present occurs in quite fresh gatherings where all around—desmids, diatoms and other algæ, infusoria and rotatoria, &c.—are in quite active and healthy condition. Hence Mr. Archer ventured to suppose, at least *ad interim*, that it could be hardly correct to relegate such forms as this, of local occurrence (and *quere* also Prof. Lankester's *B. rubescens*), and which live and flourish in seemingly only healthy environments, to one of the same *genus* as the common and universal forms of putrescent or decaying, often stinking, infusions. The long and slender little filamentary bodies combined in a manner approximately parallel, in 4's and 8's, or sometimes in 16's, within their common oblong or ovate, definitely bounded mucous matrix, might

in the present form be taken for some possibly undescribed alga, whilst the refringent "heads," in the active state, though in aspect and nature not seemingly materially different, except perhaps in size, from the similar bodies forming the joints or links in a Vibrio, &c., still, from their occupying but one extremity, would more forcibly call to mind the "spores" of certain veritable Nostochacean forms; they are sometimes detached and met with separately. This when in the active or moving state is a very agile form, incessantly darting backwards and forwards, and "poking" its way into and again out from the masses of "dirt" and heterogeneous objects around.

18*th November*, 1875.

Notice of some Desmidian forms allied to Closterium obtusum, Bréb.—Mr. Archer showed specimens of two new doubtlessly Desmidian forms closely allied to, but distinct from, that referred to Closterium under the name of *Cl. obtusum* by de Brébisson. One was long, very slender, slightly curved, and ends truncate, close to which a colourless space, without granules, beyond the medium longitudinal string of green contents. The other was considerably stouter, margin subparallel for a considerable distance, towards the ends somewhat suddenly attenuated; apices bluntly rounded, close to which a colourless space beyond the median longitudinal string of green contents, containing a *single fixed* and *still* granule. In both forms the nucleus was excentric, and placed in a lateral depression of the endochrome-mass. It will be seen that these hardly accord with the characters of Closterium. Mr. Archer thought it possible that, with some two or three others kindred thereto, they might take a place as a distinct genus close to Closterium and Penium, as well as near Spirotænia and Cylindrocystis. Before arriving at a conclusion some of the other forms in view would have to be refound and submitted to a further study.

Aulacodiscus Johnsonii, exhibited.—Rev. E. O'Meara showed a fine mounting by the Club's Corresponding Member, Mr. Kitton, of Norwich, of *Aulacodiscus Johnsonii;* in one instance the number of nodules on one valve was greater than on the other.

Structure of Petioles in genus Nymphæa.—Mr. Mackintosh exhibited transverse sections of the petioles of *Nymphæa odorata* and *N. cærulea*, along with *N. alba*, all belonging to the series with stellate cells in their air-passages, and which might therefore be termed "asteridiophorous." So far as he could make out, the only difference discernible in the petioles was in the number of secondary air-passages, which were generally *two* in *N. odorata*, from *six* to *eight* in *N. cærulea*, and from *nine* to *twelve* and even more in *N. alba*. The number of primary air-passages was four instead of ten, as in the "anasteridiophorous" series, examples of which were exhibited at last meeting of the Club.

Tetraspores in Stenogramme interrupta.—Dr. E. Perceval Wright showed examples with tetraspores of *Stenogramme interrupta*, and referred to a notice in 'Grevillea' by E. M. Holmes, in

which that writer had stated that the tetrasporic fruit had not been noticed or figured in any work published in Great Britain; Dr. Wright, however, pointed out that this was not so, but that Dr. Harvey and Miss Gifford had long since recorded it, as already fully detailed in last number of this Journal.

Another "headed" Bacterian and note thereon.—Mr. Archer wished once again to draw attention to another example of the glœogenous, still ("Zooglœa") condition of the bacterian which he had exhibited at last meeting of the Club, then both in the same and in the active moving state, and this the more especially as he desired to place side by side therewith a specimen or glœogenous group of the very same "species," "form" or "thing," which he had just noticed on a slide of Herr Otto Nordstedt's mounting, put-up for a single specimen of a Staurastrum, the bacterian being there, of course, quite unwittingly and accidentally. This was *absolutely*, and in its (granted, very few) details *precisely*, one and the same thing to which Mr. Archer had previously drawn attention. It was very pretty to see how nicely it had "mounted" and maintained its ordinary aspect. If it were met by an observer in the condition of both examples for the first time it would, without doubt, be naturally *à priori* regarded (as before mentioned) as an alga, and its bacterian nature would hardly suggest itself. Generally speaking, no heads (or "spores"?) are then visible on the little threads, but Mr. Archer had seen glœogenous specimens showing the heads more or less grown, and in different examples of different sizes. Mr. Archer was glad to be able to show the form in question (in both native and "foreign" examples!) simultaneously with another "species" to which he now drew the Club's attention, doubtless closely resembling the preceding, but yet different. Here the little threads were, microscopically speaking, considerably thinner, of a bluer tint, rigid, likewise "headed," but the heads considerably more minute. Their movements were far more lazy; the pin-like examples might be said, so to speak, merely to *totter* about. Of this form Mr. Archer had not been able to see any glœogenous state, so far as he could make out, but on the other hand he drew attention to certain slender filaments in the same gathering of great tenuity, bearing along their length little bluish bead-like bodies at even distances, distributed in pairs, suggesting the breaking up of the filament into short pieces between the beads, which would thus stand as the "heads." At other times filaments could be found in still the same gathering, showing at shorter, but likewise even, distances pairs of beads extremely minute and pairs of the ordinary size *alternating* with each other. To say the least, the moving forms, slowly wabbling about in their vacillating manner (so different from the vivacious and restless up-and-down fidget of the preceding form, though there were none of those in the free and active state to-night), were extremely like the several portions of the filament, as described, mutually detached and become independent. Their colour, especially the

heads, was of a bluish tint; in fact, they looked very like some minute "nostochaceous" joints, and Mr. Archer thought there could be little doubt but that these beads or heads corresponded to the "spores" of characteristic "Nostochaceæ," with which, indeed, the "Bacterieæ" should be associated. (It is but right to mention that these notes and views were laid before the Club prior to the appearance, in Dublin at least, of Professor Cohn's recent paper on Bacterians in the late number of his 'Beiträge,' in which similar views are expressed, and in which he now refers the various forms to their respective places in the system amongst Phycochromaceous Algæ.)

Medical Microscopical Society.

17th December, 1875.

H. Power, Esq., Vice-President, in the Chair.

Dr. Pritchard exhibited in action the new freezing machine for cutting microscopic sections, which has already been described in the 'Lancet' for December 11th, 1875.

The principle upon which its action depends is that a block of copper cooled by immersion in ice and salt will retain its low temperature sufficiently long in order to enable sections to be cut from a small piece of any soft tissue placed upon it, and which by contact with it has become frozen and adherent to its surface. If first immersed in gum water the specimens solidified better.

In the discussion that followed, Mr. Ward suggested using a metal plug in the same way as Dr. Pritchard recommended, only dropping it into an ordinary microtome tube, so as to obtain the additional advantage of a rest for the razor.

Mr. Groves thought that if the plug were hollowed so as to contain some ice and salt it would remain cold much longer.

Acari in diabetic urine.—Mr. Jabez Hogg showed a specimen of urine from a case of incipient diabetes which contained large quantities of the *Acarus domesticus*, as well as particles of indigo. Twenty-four hours after being voided he observed their presence, and up to the present time they were still alive and breeding (for they were seen in all stages of development), though six weeks had elapsed. The mycelium of the diabetic fungus had appeared and the indigo was increasing. It was possible that the animal fed on these two substances. He had only examined this one specimen, and had kept it in the bottle in which it had been sent to him, in the window all the time. He had no doubt it was the ordinary sugar acarus, and must have obtained access to the urine in the first instance by accident.

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MEMOIRS.

On the Formation *of* Blood-vessels, *as observed in the* Omentum *of* Young Rabbits. By G. Thin, M.D. (With Plate XV, figs. 1—5.)

When new capillary blood-vessels are formed from previously existing vessels in a tissue sufficiently transparent to be examined under the microscope, there is an appearance constantly observed, regarding the cause of which there is much difference of opinion. From the wall of the capillary a conical mass projects into the surrounding non-vascular tissue, and it is frequently continued into a similar mass that meets it from a neighbouring or from another part of the same vessel. Nuclei are visible at various parts of the tract thus indicated, and later a fully formed capillary is found to have been formed in it.

Golobew[1] believes that the first stage in this projecting mass is an outgrowth from a spindle-cell in the wall of the vessel, and that the new formation extends by a hollowing out of the process and a multiplication of spindle-cells, which bend over towards each other. The dark lines seen in a capillary treated by silver correspond to the line of junction of the borders of two spindle-cells.

Kölliker[2] at one time taught that a "bud" (sprossen) grew out from the wall, and either joined a similar bud from another or the same vessel, or became continuous with the spindle or stellate cells of the tissue. In the last edition of his work he acknowledges that with the present better knowledge of the structure of capillaries these views are no longer tenable.

Stricker considers that the projecting process proceeds from a stellate cell in the wall of the capillary, which anastomoses with other stellate cells in the tissue. The widening of the lumen of these processes constitutes the new vessel. This, of

[1] 'Arch. f. Mic. Anat.,' 1869, p. 49.
[2] 'Handbuch der Gewebelehre.'

course, presupposes a communication between the lumen of the processes of stellate cells and the lumen of the capillary. My information regarding Stricker's views is obtained from Klein's treatise on the omentum.[1] The last-named author adopts Stricker's view, with the addition that this excavation of branched cells "goes on both from the already formed vessel, and also isolated in the branched lymph-canalicular cells, which are in direct continuity with the endothelium of the blood-vessels."

Schäfer[2] regards the capillaries as being formed in spindle cells, absorption of part of the cell walls where the one overlaps the other being the mechanism by which a continuous tube is formed. Rouget[3] regards the projecting process as a development of the protoplasm of the wall of the vessel, which "vacuolates" and then forms a tube continuous with that of the parent vessel. Ranvier[4] regards this process as a cellular bud (*bourgeon cellulaire*), which gradually hollows out as it forms.

Thus the same appearance is regarded by Stricker and Klein as an enlarging process of a stellate cell; by Golobew as an outgrowth of a spindle-cell; by Rouget as an outgrowth of the protoplasm of the wall of the vessel; and by Ranvier as a cellular bud. Kölliker used a similar term (*sprossen*) as applied to the same object, without defining it further. Kölliker was the first to describe this projecting mass as being continuous with the stellate cells of the tissue, in which Stricker and Klein follow him. Golobew, Rouget, and Ranvier, on the other hand, state that they have never seen it become continuous with a stellate cell.

Ranvier draws a sharp distinction between the growth of a capillary from a pre-existing vessel and the independent development of capillaries in the connective tissue which, after they are fully formed, join the other blood-vessels.

Klein describes, as we have seen, an independent formation in stellate cells which "vacuolate," and Schäfer by the hollowing out of spindle-cells.

To return to the projecting mass from an already formed capillary. This has been most frequently observed and figured, as seen in the tail of the tadpole. It is easy of observation, and there can be little doubt that the different observers saw exactly the same appearance, as is besides clear enough from their figures. Since the same appearance has

[1] 'The Anatomy of the Lymphatic System,' i, p. 61.
[2] 'Proc. Roy. Soc.,' No. 151, 1874.
[3] 'Arch. de Physiol.,' 1873, p. 603.
[4] 'Arch. de Physiol.,' 1874, p. 429.

been so variously interpreted, it follows that there can be nothing in its objective characters which is distinctive, each observer deciding for himself as to its nature in accordance with his preconceived ideas.

I made, some time ago, a special study of the development of blood-vessels in fœtal and in inflamed tissue. I found that when a mouse fœtus, at the stage of development fitted for the study, was placed in half per cent. solution of osmic acid, and allowed to remain some hours in the solution, portions of the integument, the cranial bones, and other tissues, could be placed entire under the microscope, and examined in very favorable circumstances. Such preparations are particularly well suited for an examination of the nature of the conical mass which projects from the previously formed vessels. The results which I obtained in this manner can be stated shortly. The conical mass is composed of amorphous matter between the bundles of connective tissue. It refracts light differently from the connective tissue itself, and stains differently from either the connective tissue or any cellular element observable in connection with that tissue or with the vascular wall. It contains no nucleus, and its form is at no stage like that of any known cell or part of any known cell. I can, therefore, see no foundation for supposing it to be cellular. The large nucleated blood-corpuscles of the fœtus can be seen wedging their way into it through the vascular wall, a thin, narrow part of the blood-cell being within the mass, and a large spherical part of it being still within the vessel. It is fair to assume that an opening through which a blood-corpuscle can force its way will permit the fluid plasma of the blood to pass, and into the space represented by this appearance the blood-plasma must have been passing in greater or less quantity. It is in this plasma, rich in fibrinogenic material, that I find an explanation of the conical mass which projects from the capillaries.

In osmic-acid preparations spindle-cells with large, sometimes multiple, nuclei can be seen in the walls of the space in which the mass is contained.

In fresh preparations, or preparations obtained by many of the commonly used reagents, the nuclei alone of these spindle-cells are seen, and the appearance is apt to suggest the idea of a cellular structure. Osmic acid possesses the quality of enabling us to see the spindle-cell as well as its nucleus, and thus strips the space in which the cells lie, and into which the blood forces its way, of all claim to be regarded as cellular in its nature.

In my studies on the fœtal tissue of the mouse, I did no

succeed in isolating the developing epithelium of the young capillaries, but in the new blood-vessels formed in the inflamed cornea I had better success. When the cornea had been treated by osmic acid and sections disentangled with needles, the new vessels were often found to be indicated by columns of blood-corpuscles, which were adherent to isolated bands of corneal tissue. When the surface of these bundles on which the corpuscles lay was gently teased, cells could be isolated, showing all possible degrees of transition, from the colourless blood-cells to that of a fully formed epithelial cell. The first stage of development consisted in the formation of a large round nucleus. As the cell enlarged, the granular appearance rapidly disappeared, and the cell could only be distinguished by the contours of the nucleus and of the cell.

These results have been already communicated to the Royal Society, in a paper now in the possession of the Society, of which an abstract has been published ('Proc. Royal Soc.,' No. 160, 1875).

I have, since that memoir was written, followed M. Ranvier's studies on the development of blood-vessels in the omentum of young rabbits, and the principal object of the present paper is to give the results at which I have arrived, after what may be described as an experimental criticism of M. Ranvier's investigations.

It is only necessary to follow M. Ranvier's simple directions to obtain preparations that perfectly correspond to his drawings, and the most important points which he illustrates and discusses may be seen by the simplest of his methods.

A piece of the free omentum, at the pyloric end of the stomach of a rabbit a month or five weeks old, is placed in Müller's fluid immediately after the animal is killed, and after twenty-four hours is gently brushed on both surfaces in the solution, and then washed in distilled water. It is finally stained with picrocarminate, and immediately examined in glycerine. The milky patches (*taches laiteuses*) of Ranvier[1] —the lymphatic nodules of Knauff ('Centralblat,' No. 36, 1867), lymphangial nodules of Klein—can then be seen with the naked eye as small red spots, and when examined under the microscope the cellular elements, with more or less of other appearances, can be observed.

In examining a delicate tissue like the omentum there are several points that are of importance. The membrane should not be allowed to shrink, nor be forcibly stretched, nor to

[1] As these patches when observed in the fresh omentum examined in peritoneal serosity appear as opaque slightly raised spots, Ranvier has designated them *taches laiteuses*. I prefer this term, as it implies no theory.

become dry, nor, I may add, come in contact with water, except where that is unavoidable, as after being stained. Ranvier fulfils these conditions by his process of *semi-dessication*, for the details of which I refer the reader to his essay, or the 'Traité technique d'Histologie." I adopted the following method, which I found to answer well. Two pieces of mica, about an inch long and half an inch broad, are placed one over the other, and a hole punched through both very near one end. Small notches are made in the edges of the pieces of mica before and behind the area of the holes. One of the pieces should be thin. Whilst an assistant gently raises the free edge of the omentum the thicker piece of mica is slipped under it, and the thin piece is laid over it so as to fit the under one accurately. Thus in the area of the holes in the mica a portion of omentum is fixed. The pieces of mica are held firmly together by forceps, and the omentum cut round their edges with scissors. They are then tied firmly together with strong thread well waxed, which is fixed by the notches. This done, the mica is cut across behind the posterior ligature, the portion that previously served as a handle being no longer required.

By this procedure I obtained a portion of omentum in its natural condition, and which could not shrink. I could move it from one solution to another, and by suspending it in a fluid with forceps I could brush both surfaces. Finally, when I wished to mount it for examination, I laid it on a large drop of glycerine, and putting another drop on the upper surface, I covered the whole with a cover-glass. To make the preparation permanent it was only necessary to run Brunswick black round the edges. If the upper layer of mica, with the addition of a cover-glass, is too thick to permit examination with a high power, it can frequently be removed without disturbing the membrane, which, after it has been soaked in glycerine, has little tendency to retract.

The time occupied in securing the omentum between the mica plates is more than regained by the rapidity with which all the other manipulations can be afterwards executed. The preparations made in this way were more satisfactory than those obtained by any other method which I tried. I owe the happy suggestion of the mica plates for this purpose to Mr. Ewart.

The mica squares with their enclosed omentum were treated by half and tenth per cent. solutions of osmic acid, by half per cent. solution of chloride of gold, by logwood-glycerine, by Müller's fluid, and by solution of purpurine according to Ranvier's formula. Those treated by Müller's

fluid were pencilled and then stained in carmine, picrocarminate, logwood, and purpurine. Preparations previously injected with Berlin blue were placed in Müller's fluid and picrocarminate.

Before explaining what I observed in preparations thus variously treated, it will be convenient to return to M. Ranvier's paper. M. Ranvier describes in the omentum a network of cells which are destined to form new capillary vessels. He therefore designates them "vaso-formative cells," and especially insists that they are distinct from the cells of the connective tissue; the cell being, in fact, *sui generis*. Examined in peritoneal serosity these cells are described as "irregularly branched, finely granular, and highly refractive. Their size, and the number and arrangement of their prolongations are very variable. The branches often anastomose with each other to form a network. In a given milky patch into which the blood has not yet penetruted this network has the form of a capillary network." He has not been able to determine the number of cells in a network, but believes that it is not considerable, and that one cell may form a whole network.

In preparations which have been treated by Müller's fluid, pencilled and stained in carmine, Ranvier recognised that in the midst of the granular protoplasm which forms the mass of all the branches of the vaso-formative network, elongated cylindrical nuclei are situated, whose long axis corresponds to that of the branch which they occupy. These nuclei are highly refractive and stain deeply with carmine.

The questions which I endeavoured to answer from a study of my preparations were: Does the appearance described and figured by Ranvier constitute a definite structure to which the term "cell" is applicable? and what relation does it have to the formation of new blood-vessels?

In specimens stained in carmine and picrocarminate it is possible to obtain preparations which correspond exactly to Ranvier's figures of the "*cellule vaso-formative*," but in my experience appearances accurately corresponding to the figure are rather the exception than the rule. Various modifications of the appearance having, however, obviously the same relation to a structural peculiarity of the tissue are always found. Let us analyse the "cellule vaso-formative" as described by Ranvier in stained preparations. There is in an almost colourless ground substance a branched pale red figure, and at different parts of the figure more deeply stained nuclei. There is nothing in this appearance itself that would indicate that the figure represents a cell.

M. Ranvier has himself taught us that in a transverse section of tendon, for example, the presence of nuclei in a ramifying network does not constitute the network a system of branched cells. In the omentum the question hinges upon our being able to discover the nature of the cells to which the nuclei visible in the appearance we are discussing belong, and this I believe I have been able to do. Even in carmine preparations it is sometimes possible to detect in the walls of the branching figure that beyond the ends of the elongated nuclei there is a scant, deeply stained, finely granular substance which narrows to a point, and beyond this is continued into an unstained fibre which differs in its refractive power from the surrounding tissue, the distinguishing characters of a spindle-cell being in fact unmistakeable. It is possible in such a preparation to resolve all the nuclei visible into spindle-cells which lie in the wall of the figure The figure itself becomes thus reduced to a ramifying stained, non-nucleated mass, and as faintly stained, narrow prolongations of it can often be traced for a great distance, I must add, of indefinite extent.

The peculiarity of carmine preparations is that the mass constituting the so-called *cellule vaso-formative* stains uniformly in all its breadth, rendering the detection of the spindle-cells very difficult. To show clearly the nature of these appearances we must have recourse to other dyes.

If the mica square is placed in a half per cent. solution of osmic acid for twenty-four hours and then stained in log-wood solution, we find the appearance is very materially altered. The spindle-cells are deeply stained, the ramifying figure very faintly or not at all. It is sometimes, indeed, distinguished by its being less stained than the surrounding tissue. Instead of a red branching figure containing nuclei in an uncoloured ground, we have in a faintly coloured ground a colourless figure, in the walls of which are deeply stained spindle-cells. The preparation is dotted all over with a great number of large oval nuclei, but these have no special relation to the figure we are discussing. The lymph-cells are stained a uniform deep blue.

If the mica square is placed in solution of purpurine for twenty-four hours, and is then examined in glycerine, we have preparations that at first sight seem to resemble those coloured by carmine. They differ from them, however, in some important particulars. The branching figure is very faintly stained, the spindle-cell is more deeply stained, and the lymph-cells are conspicuous bodies. Occasionally a lymph-cell, perfectly to be distinguished from the large oval

nuclei by its round form and well-marked small multiple nuclei, is seen in the figure. In short, what was evident enough in the carmine preparations, and confirmed by the osmic-acid and logwood preparations, is still further confirmed by the purpurine, namely, that the "cellule vaso-formative" of Ranvier is not a cell, but a ramifying space of varying extent.

There is nothing of a special nature in these ramifying spaces, either in the milky patches or in other parts of the membrane, except their breadth. They exist to a less developed extent over the whole omentum. In the omentum of a new-born kitten they may be observed scattered over it in a discrete form. They are simply spaces between the bundles of connective tissue, such as occur in all forms of that tissue, the special appearance they present in a given instance being determined by the arrangement of the bundles.

This view is further supported by treatment with logwood-glycerine. In order to obviate the destructive effects of water on delicate tissues, I conceived the idea of colouring glycerine by dropping into it a sufficient quantity of a concentrated solution of the dye, and placing the fresh tissue which I wished to stain in the coloured glycerine. The effects obtained by its use differ in many respects from those observed when an aqueous solution of the dye is used. When the mica square is placed in logwood-glycerine for twenty-four hours, then washed in pure glycerine and examined, the membrane is seen to be permeated by an anastomosing blue network, of which the distended spaces ("cellules vaso-formatives") are seen to form a part. Few nuclei are stained, even the lymph-cells are not much coloured, and the connective tissue is colourless, but all the wider spaces of the tissue are indicated by a faint blue colour.

In fig. 3 the effect of purpurine on the spaces is illustrated. The space, a spindle nucleus and part of the protoplasm of the spindle-cell, are to be distinguished, and a well-marked lymph-corpuscle is seen at the wide end of the space.

What I have been able to make out regarding the connection which these spaces have with the formation of blood-vessels is exactly in accord with Ranvier's observations, if we substitute for his idea of a cell that of a space. But I have further observed that there is a peculiar development of spindle-cells in connection with this new formation, in regard to which that author makes no remarks, and which

is analogous to what I had previously observed in the mouse fœtus and in the inflamed cornea.

One of the first indications of the distension of the inter-fascicular spaces which precedes the growth of a new capillary is the presence in them of large spindle-cells. The nucleus is very evident in deeply stained carmine preparations, not so much because it is stained as it is because it contrasts with the more deeply stained cell protoplasm in which it lies. In osmic-acid preparations, stained subsequently in logwood, the spindle-cell protoplasm stains very deeply, obscuring the nucleus. There is accompanying the enlarged spindle an occasional (it may be constant) escape of nuclear bodies, which leaves a corresponding cavity observable in the cell. A similar escape of small rounded bodies with corresponding cavities is observable in the lymph-cells in the neighbourhood. The distended space, enlarged spindles, and vacuoles in one of the spindles, and some of the lymph-cells in a milky patch, which is ripe for a connection with the general circulation, is shown in fig. 4.

I have not traced the further changes in these enlarged spindles. They do not remain in the swollen condition, but whether they shrink to the minute spindle element of the mature tissue or disintegrate and are absorbed is a matter for further observation.

Enlarged spindles may precede the distension of the inter-fascicular spaces, and may alone indicate the direction in which a growing capillary will develop. Fig. 5 illustrates this change. At one end of the figure the extent to which a capillary has developed is seen, and on the left a line of enlarged spindle-cells between which and the capillary fine streaks indicated the line by which they would have been subsequently connected. The distance in the preparation between the spindle-cells and the capillary was double what I have been able to show in the figure. The capillary and the spindle cells were stained a deep red, the tissue of the omentum being unstained, and there being no similarly stained spindle-cells except in connection with growing vessels.

The contents of the inter-fascicular spaces which are enlarging previous to being connected with the blood-vessels differ from the lymph fluid normally present in the interstices in so much as they stain more or less deeply in carmine. It is this property that has led to their being considered cellular. This deep staining in carmine affects the blind ends of the newly formed capillaries as well as the contents of the spaces. Taking this in conjunction with

the fact that the spaces which are shortly to become connected with the vessels colour more deeply than the other spaces in the tissue, I make the hypothesis that the substance that stains is formed in the blood plasma that has escaped from the vessel. I assume that permeability of the vessel to the fluid elements of the blood has already taken place, and that we have consequently present in the widened interstices of the tissue in which it accumulates a plasma which differs from the ordinary lymph fluid. This plasma is the forerunner of the blood current, and when it has sufficiently distended the communication between the spaces and the interior of the vessel, the circulation is practically established.

These facts receive confirmation from certain injected preparations, such as that which is partly shown in figure 1. In this preparation the vessels were completely injected with Berlin blue, the mica square placed some hours in Müller's fluid, stained in picrocarminate and mounted in glycerine. In most of the milky patches the vascular network was complete. In the patch shown in figure 1 the vessels had not formed. The substance of the patch differed, both from that of the patches in which the vessels had formed, and from the non-vascular parts of the membrane, by showing a close network stained by the picrocarminate of such exceeding abundance and intricacy that the attempt has not been made to reproduce it.

The size of the patch in the figure shows the extent of the staining, and also the distinct manner in which it was separated from the unstained membrane. There was thus present in the interstices of the patch, even in the smallest of them, a substance that stained in picrocarminate, and this substance had disappeared from the interstices of those patches in whose larger spaces capillary vessels had fully developed.

By looking at the same figure it will be seen that the injected mass left the capillary at a certain point, and after a short straight course penetrated the larger interstitial spaces of the patch, whose characteristic arrangement can be traced by the coloured injection. There was thus a communication between the lumen of the capillary and the interstitial spaces before the latter were permeable for the whole of the contents of the blood-vessels.

I have purposely avoided discussing the histology of the omentum, except in so far as it bears directly on the subject of development of blood-vessels; but in order to show the difference between the spaces and cells I have represented two branched cells in figure 2, and the nuclei of these cells

may be compared with the nuclei of the flat cells in the interior of the membrane, which are shown beside them. The branched cells shown are in an early stage of development. Their further development does not consist in increase of size, but in absorption of the protoplasm which surrounds the nucleus and the conversion of the stained granular-looking processes into delicate glistening threads, which are incapable of staining, but which may be sometimes recognised in osmic-acid preparations examined in solution of acetate of potash.

The conclusion to which I have therefore come is, that the "cellules vaso-formatives" of Ranvier are spaces in the omentum, to which I submit the term "cell" is not applicable.

The development of blood-vessels takes place by an escape of, first, the fluid, and finally of the formed elements of the blood, from the vascular system into those spaces. The establishment of the blood current is speedily followed by the formation of a membranous wall around the current, which is impermeable for an injection mass or the blood, and the process is complete. My previous observations on the growth of vessels in fœtal and in inflamed tissue are therefore in harmony with those made on the omentum, and recorded in this paper.

On the Structure *of* Muscular Fibre. By G. Thin, M.D. (With Plate XV, figs. 6—14.)

In order that the value of the preparations which it is the object of this paper to describe may be properly estimated it is necessary to refer the reader to a paper I published in 'The Edinburgh Medical Journal' for September, 1874, *On the Minute Anatomy of Muscle and Tendon*, and especially to the figures by which it is illustrated. For the sake of completeness I will, however, give here a short summary of the results of my investigations up to the time when that paper was written.

In stained transverse sections of muscular fibres of the frog, which had been carefully hardened in weak solution of chromic acid without any further hardening by alcohol, Cohnheim's fields were seen to contain about twelve to fifteen fibrillæ. The fibrilla was recognised both in chromic acid and in gold preparations as an exceedingly fine cylin-

drical thread of definite and uniform size and appearance, and in gold preparations exposed to light in acidulated water an intermediate darkly-stained substance was seen to adhere to the fibrilla.

Muscular fibres of the frog were sealed up on an object-glass in mixtures of half per cent. solution of chloride of gold and acetic acid in varying proportions, and the changes which took place observed daily. Amongst these changes there were two distinct kinds of appearance seen. In some instances the mass composing the muscular fibre became for the most part nearly invisible, and in it parallel rows of spindle elements became prominent. These consisted of a longitudinal series of oval swellings connected by a round well-marked fibre. The independence of these spindle elements as regards the substance of the fibre was established by their being often seen to project beyond it. In another set of preparations an immense number of rounded discs were scattered over the field, singly and combined in circular groups. These came from the end of the fibre, which was seen to be full of them, the appearance being likened to the open end of the cornucopia. The fibre, which formed the subject of illustration in the paper referred to, when looked at some weeks later was observed to have been divided into these small discs throughout, the form of the fibre, however, being retained except at the ends.

When the muscular fibes of the frog were deeply stained by the action of chloride of gold and subsequent exposure to light in acidulated water, and finally kept for six to twenty-four hours at the temperature of 100° Fahr. in strong acetic acid, the muscle substance could be manipulated by needles, and there could be isolated from it portions of a network more or less large. This network was composed of exceedingly fine fibres, and its meshes accurately corresponded to Cohnheim's fields. The isolation and the independence of the minute fibres composing it were complete. In the central points of the meshes a minute spot had been stained by the gold solution, and scattered over the network were single larger stained patches from which fibres radiated that anastomosed with those of the network. None of the portions isolated were larger than the area of a transverse section. The deeply stained larger patches had the form and tint of the branched cells of connective tissues when these are similarly treated. In fibres treated by gold solution and acidulated water I had isolated elongated narrow nucleated cells lying amongst the fibrillæ. In fibres which had been two or three days in gold solution, then mounted in a mixture of gold solution and

acetic acid, the acid being in excess, and finally exposed to bright sunlight, the outlines of long narrow cells and of broad oblong cells were visible. (I subsequently succeeded in isolating one of the broad cells in a fibre treated in the ordinary way by gold solution and subsequent exposure to light in acidulated water.)

In gold preparations the end of a fibre had been sometimes observed to be composed of a number of well-defined compartments, which when they shrunk towards the sarcolemma after being acted on by chloride of gold, produced a central canal of considerable dimensions.

I had thus demonstrated in muscular fibre by the most satisfactory of all methods, namely, isolation, the existence of flat cells, of a network connected with central cellular protoplasm, and of parallel rows of spindle elements. I had obtained confirmatory evidence of the existence of the appearance known as Cohnheim's fields, and had produced artificially discs which corresponded to transverse sections of Kölliker's *muskel-säulchen*, and which also corresponded to Cohnheim's fields. I had seen reason to believe in the existence of the fibrilla as a separate entity and not as the production of reagents. I could thus find evidence of fibrillæ, of cylindrical bands parallel to the long axis of the fibre which I designated primary bundles (of fibrillæ) to mark their analogy with similar structures in tendon and connective tissue, and of groups of these, which for a similar reason I designated secondary bundles, the whole forming the contents of the sarcolemma. Between the primary bundles were rows of spindle elements and the existence of a network corresponding in size and arrangement to Cohnheim's fields, justified the assumption that its meshes embraced the primary bundles. It was assumed from their position in teased preparations that the flat cells were applied to the surfaces of the bundles.

I have in the interval that has elapsed since the paper referred to was written obtained valuable confirmation of the accuracy of my views. In June, 1874, whilst engaged in the investigations already recorded, I treated fresh muscular fibres of the frog with logwood solution and glycerine in various combinations. The fibres were sometimes rapidly stained with a strong solution and then placed in glycerine; they were sometimes placed immediately in glycerine, and logwood solution was drawn through the preparation by blotting-paper suitably placed at the edge of the cover glass, the excess being removed by fresh glycerine being drawn through in a similar way.

The object of these manœuvres was to stain the nuclei and, if possible, the cells in the substance of the fibre without subjecting them to the destructive influence of watery solutions. I had already at that time acquired the conviction which has been since much strengthened, that one reason why the delicate flat cells in the tissues have not been seen is that they do not resist the action of water or alcohol.

The muscular fibres so stained showed many nuclei, a greater number than I had seen by any other mode of preparation except that by warm saturated solution of caustic potash. But I was foiled in endeavouring to obtain by this method preparations showing more than the existence of a great number of nuclei on account of the difficulties presented to manipulation by the sarcolemma, and I came to look on the sarcolemma as a great obstacle in the way of a better knowledge of the structure of muscle. That obstacle has, however, been now overcome.

Amongst the stained fibres one of the preparations was preserved on account of its beauty and the number of stained nuclei. It consisted of several fibres teased so that their relation to each other was no longer observable. It was stained and mounted in May, 1874. In August, 1875, the logwood staining had become diffused, the nuclei being obscured, the preparation consisting of a number of deep blue fibres, uniformly coloured, in which the individual elements could not be distinctly observed. The cover glass was carefully removed and a large drop of pure glycerine was placed over the fibres. A cover glass was not put on, but the preparation was sheltered from dust and laid aside. In February, 1876, I teased the fibres. The change effected by the long maceration was at once evident. I found that each fibre could be split into three or four divisions, which were so easily separated from each other that when the end of a fibre was fixed on one side by a needle and on the other side by another needle, it was only necessary to separate the needles from each other, the points being kept fixed, to cleave the entire fibre. When the cleaving was observed under a dissecting lens it was seen to take place so uniformly and evenly that it became evident that it followed a course determined by a structural pecularity of the fibre. One of the parts into which a fibre could be thus divided usually corresponded in breadth or diameter to that component part of a fibre which I had, when seen indicated in gold preparations, designated a secondary bundle, and I shall for the sake of distinctness apply that term to it in this paper.

A secondary bundle was sufficiently transparent to show

that it was formed by a number of narrow parallel cylindrical bands corresponding to what I had designated a primary bundle and to Kölliker's *Muskel-säulchen*. I shall continue in this paper to use in reference to them the term primary bundle. When a secondary bundle was teased a primary bundle could occasionally be isolated to a considerable length, and the only reason why there was any difficulty in isolating them was the size of the points of the needles as compared with the narrowness of the structures which had to be manipulated. There was no other than the mechanical difficulty of manipulation to prevent every muscular fibre being completely separated into isolated primary bundles, and with fine needles, a good dissecting lens and sufficient patience, this would have been possible. I will return to what I believe to be the cause of this extraordinary change in the physical qualities of the fibres, but will first describe the histological characters of the different elements which were thus made available for study.

In the undivided fibre an indication of the secondary bundles could be detected where it was flattened, and the primary bundles were indicated by an arrangement of nuclei in parallel lines, the number of nuclei visible being enormous. In fig. 10 a fibre is shown as seen by a low power (No. 3 Obj. of Hartnack); at one end the fibre is seen unbroken, whilst at the other it is seen broken up into three parts. Fig. 11 shows more highly magnified the divided end of the same fibre and the nuclei with which it is filled. In order to give a still clearer idea of the number of nuclei fig. 12 has been added.

Since the primary bundles could also be separated from each other the objection might fairly be taken to the secondary bundles that they represent parts of the fibre separated fortuitously, and in some instances this may have been the case. But against this view as applicable to all the so-called secondary bundles is the uniform ease with which a fibre could always be separated into several parts of fairly equal size, and the fact of the indubitable arrangement of the muscular fibres of the frog in secondary bundles which I had shown by the action of chloride of gold. (See the 'Proc. Roy. Sec.,' No. 155, 1874; Plate XI, figs. 26 and 28.)

In the preparations I am now considering the most important individual element is the primary bundle. The secondary bundles and the fibre itself are simply collected groups of these. By no previous method of preparation has this important element been so completely brought within the scope of observation.

To begin with, its individual existence is placed beyond the reach of rational scepticism. The appearance it presents is distinct. The breadth of all the bundles which have been isolated is uniform, and corresponds to the breadth which is indicated when they are in their natural position in the larger bundles. The individual primary bundles can be easily traced in the secondary bundles. They never join or become confounded with each other. When isolated they were seen to be unstained, but the remains of a shreddy film of membranous substance stained by logwood was sometimes adherent to their surface. The surface of the primary bundle itself was even and unbroken. The action of the dye is evidently much dependent on exposure to light, as when the preparations after being laid aside for a week were again examined, the substance of the bundles was in some instances stained a faint blue. This increased effect of the dye on elements which had been spared when protected in the substance of the fibre makes the permanence of the preparations doubtful. The breadth of the primary bundles is slightly less than the diameter of a human red blood-corpuscle.

The more the bundles were disturbed by efforts to isolate them the more they were stripped of the nuclei which are applied to their surface; but in some of them the arrangement of the nuclei can be well seen even after isolation. The cells to which the nuclei belonged are in some instances preserved. Figs. 6, 7, and 8 represent the primary bundles and the nuclei adherent to them. In fig. 8 two narrow cells are seen entire. The mode of application of the cells is indicated by the nucleus being observed to double round the bundle.[1]

The nuclei are much better preserved than the cells, and can be observed to be distinctly of several sizes which would indicate cells of different size or form. Two distinct forms of cells have been seen, namely, the narrow elongated cells shown in fig. 8 and the cells shown in fig. 9 (*a*).

The cells shown in fig. 9 (*a*) are broad, square cells, precisely similar in form and arrangement to the cells described by Ranvier in tendon, with this difference—that they are smaller than the tendon-cells are in the animals in which they are usually studied. This resemblance to the tendon cells is shown further in a striking manner by the position of the nuclei. In the tendon-cells the nuclei are not in the centre

[1] The fold thus produced on the surface of the nucleus (*crête d'empriente* of Ranvier) has been already described in the unbroken fibre by Dr. Weber, of Paris.

but at one end of the cell, and are so arranged that pairs of cells have the nuclei in contiguous ends; so that the nucleus of one cell is very near that of the other. When in the muscular fibre a sufficient number of the corresponding cells have been preserved to enable the point to be noticed, it is found that the arrangement of nuclei in contiguous cells is the same as in tendon. This peculiar arrangement of the nuclei is shown at *a* in fig. 9.

The square cells are sufficiently broad to cover several primary bundles, and from the position in which I have found them I believe that they invest the secondary bundles. In their form and arrangement I find an analogy much strengthened which I formerly pointed out between the secondary bundles in a muscular fibre and the bundles of tendon which are invested by the cells described by Ranvier.

On the surface of some of the fibres remnants of the sarcolemma are visible, distinguished by large rounded nuclei, larger than any of the nuclei observed in the fibre. In one preparation I found the sarcolemma almost entire. It is represented in figs. 13 and 14, as seen by the No. 3 and No. 7 objectives of Hartnack. I was at first in doubt whether the appearance shown was not that of a part of the *perimysium internum*, but I decided in favour of its being the sarcolemma on account of its resistance, freedom from folds or wrinkles, and from a peculiar and distinctive aspect which I find it difficult to describe more closely. The sarcolemma, as seen in the drawing, shows the size of the original fibre. It contains, however, only a part of the fibre, the other parts having been probably extracted in the process of teasing. Be that as it may, this appearance, which I have only observed in one of the preparations, is sufficiently interesting to be reproduced, and I have therefore shown it as seen by a low and a higher magnifying power.

When these fibres were observed in 1874 they showed, more or less distinctly, the ordinary transverse markings. After maceration every trace of transverse marking had disappeared, and although the easy dissection of the fibres would have afforded a favorable opportunity of observing the minute network I had isolated by other methods if it had survived, there was none of it visible. I am disposed to attribute the remarkable change by which the fibre could be divided into the bundles which compose it partly to the softening of the sarcolemma, but partly also, and perhaps chiefly, to the destruction by prolonged maceration in glycerine of this transverse network, and I am compelled

to associate the transverse markings with the existence of this network without attempting to explain the connection between them more definitely.

The spindle elements had also completely disappeared.

As bearing on this matter I may remark that in the muscular fibre of a mouse which had been treated by chloride of gold solution and then sealed on an object-glass in diluted acetic acid, I found the following change after the preparation had been laid aside for more than twelve months. On one part of the fibre the transverse markings had persisted, and were more than usually well marked, the fibre at that part having an appearance as if it were bound with hoops, each transverse marking resembling one hoop. As far as the hoop-like arrangement persisted the fibre was small and of uniform calibre. At the other end there were no transverse markings, the fibre was fibrillated, and from a third to a half broader than where the transverse markings had remained. The last of these markings consisted of a distinct ring that seemed to pass round the fibre, and immediately beyond it the fibres spread out as if freed from a constriction. (The appearance so produced may be familiarly illustrated by likening it to that of a birch broom). In this fibre the connection of the transverse markings with a binding together of its constituent parts is unmistakable.

In the paper published in the 'Proceedings of the Royal Society,' No. 155, 1874, I described and figured appearances produced in muscular fibre by saturated solution of caustic potash, according to the method afterwards more minutely described in the January number of this Journal. In frog muscle which had been subjected to the solution at a temperature of 107° Fahr., the solution being allowed to cool, I have lately several times observed fibres in which the primary bundles were as clearly defined as in the preparations figured in the plate which accompanies this paper, and I have also several times seen the fibrilla in such preparations perfectly isolated. Success in these respects is in potash preparations exceptional, and was accidentally obtained by observing fibres which had adhered to other tissues on which I was operating.

A few words regarding the logwood solution may not be out of place. It is different from the solution prepared from crystallized hæmatoxylin according to the formula of Böhm. Dr. Klein gives a formula for its preparation at p. 378, vol. xiii (new series) of this Journal, which he states is similar to a method prescribed by Professor Arnold. I have not succeeded in verifying the reference to Professor Arnold's paper.

Exner, in his *Leitfaden bei der mikroscopischen Untersuchungen*, Leipzig, 1873, p. 64, directs that logwood chips are to be boiled in a concentrated solution of alum, or that the wood should be boiled in water and alum added to the decoction until it becomes of a fine blue colour. I have not been able to discover who first used the logwood as distinguished from hæmatoxylin, nor what the original formula was. Possibly the chips may succeed with more certainty than the extract. After having previously obtained an excellent solution with the extract I have lately had difficulty in obtaining an extract that would yield a good dye, and as I find other observers have found the extract bought from druggists equally unreliable, I may save trouble to some readers of the Journal by mentioning that a good logwood dye is sold by Mr. Martindale, chemist, New Cavendish Street, London, W.

I have to express my indebtedness to Mr. Ewart for the execution of the drawings in Plate XV.

An Account *of the* Recent Researches *into the* History *of the* Bacteria, *made by, and under the direction of,* Prof. Cohn.[1] By F. Jeffrey Bell, Exhibitioner of Magdalen College, Oxford. (With Plate XX.)

In the April number of the 13th volume of this Journal (1873), an account was given of the first series of observations on Bacteria which Professor Cohn had published in his valuable 'Beiträge zur Biologie der Pflanzen' (Heft 2); in the 3rd Heft, published last year, the subject is continued by Cohn, in addition to whom Eidam, working in his laboratory, gives an account of certain experiments made on these interesting organisms. Since the publication of the previous paper the discussion concerning spontaneous generation has changed in aspect; Dr. Burdon Sanderson has given an account of several Bacterioid forms, in his Reports to the Medical Officer of the Privy Council, and Professor Ray Lankester has called the attention of readers of this Journal to the subject by his very interesting account of "a peach-coloured Bacterium." To the wide interest exhibited in these forms Cohn briefly refers, but mentions at greater length the work of Billroth, published by George Reimer, of

[1] 'Beiträge zur Biologie der Pflanzen,' 3es Heft, 1875.

Berlin, in 1874.[1] I shall try to give in the following pages an account of these two new papers, but must refer all who are really interested in the matter from whatever point of view, philosophical, botanical, or pathological, to the originals themselves, assuring them that diffuseness will not be found to be a characteristic of this memoir. Notwithstanding Billroth's work, Cohn holds firmly to his original position, that the Bacteria should be arranged in genera and species, and not united into a single polymorphous species—*Coccobacteria septica* (Billroth), as has happened to all the forms described by him, with the exception of *Spirillum and Spirochæte*, under the hands of Billroth. Readers of this Journal will remember that Mr. Lankester speaks of *B. rubescens* as a Protean species. Against such a view Cohn argues that the changes of form are not fully proved, nor does he think that such a way of looking at these organisms will be as conducive to progress in our knowledge as his more objective method. With so much by way of introduction we will proceed to the consideration of the various forms which Cohn examines, one by one; it will I think conduce to clearness, if we follow his example.

1. The first examined is the *Ascococcus*, studied by Billroth. In the careful observation of the Bacteria-films found in water, containing muscle in a state of decomposition, or on hydrocele, and such like fluids, Billroth noticed a certain grey or greenish-grey organism, rounded in contour, of a knobbed or cylindrical form; the scum which they produce may attain 0·5 mm. in thickness, and forms, on account of its circumscribed position in jars, and such vessels, distinct folds; it finally sinks to the bottom of the vessel as a flocculent precipitate. These folds are caused by "a peculiar vegetative form of the coccos," which Billroth calls *palmelloid*. If embedded in "*coccoglia*" Ascococcus forms spheres or cylinders of Micrococcus, united into colonies, and held together by a remarkably fine glia; around such a scum no membrane has ever been observed, notwithstanding its definite outline; but the presence of it is to be considered as probable; and it is, indeed, remarkably thick in fluids containing beef, with sugar.

The history of this form is, according to Billroth, of the following character: several large cells form, by transverse division, long, cylindrical, and spiral tubes, in which appear chains of cocci by longitudinal division, and branching; these repeated branchings produce very small (micro)

[1] 'Untersuchungen über die Vegetationsform von Coccobacteria septica,' &c.

cocci; when this has gone on for a certain time, micrococci escape by the bursting of one side; and the envelope which is left is seen to have a double contour, and is easily recognisable by its greenish-brown colour. One remarkable form of this genus, which Billroth calls *A. parvus*, has been found by him in water containing flesh; it is a pale finely-granulated ball, not unlike an unnucleated lymph-corpuscle, which swims about by means of cilia, like the contractile Myxomonads, and soon, developing long processes, comes to resemble a Myxamœba; this form, however, Billroth regards as the encysted spore of a Myxomycetes (perhaps Æthalium septicum), while the micrococci within it have been simply eaten; if this be Ascoccocus—and Cohn thinks that it is not a Bacterian form—it is clear that Billroth has described two things under one name; the other form Cohn has, however, found, and describes as *Ascococcus Billrothii*.

2. This form he observed, not in putrescent fluids as did Billroth, but by the examination of air collected by a new process which he describes in this paper, and which is of sufficient importance to detain us for a short time. Being busied, he says, in the beginning of 1874, with the important question, as to whether germs of Bacteria capable of development under normal conditions are to be found in the air, he invented—after rejecting the processes of Pasteur and Pouchet—a method by which the Bacteria-germs, if present, are passed through a solution in which they are able to develope. Air was passed by means of an aspirator through a series of wash-bottles, which were filled with twenty grammes of his "normal fluid," the composition of which he gave in his previous essay. Two large glass bottles, each of which held ten litres, and which were made air-tight by bungs served as the aspirator; through each bung passed two glass tubes, bent at right angles, one passing to the bottom of the bottle, and the other reaching just below the bung; one bottle then being filled with water, and placed at a higher level than the other, and the tubes united by caoutchouc piping, the desired current was produced. The upper bottle was now united by caoutchouc piping to the series of wash-bottles, and the air passed in, in bubbles. When the lower bottle becomes full, it changes place with the upper; and this change, with the consequent alteration of the piping, is the only trouble to which the experimenter is put by this method; while the volume of air which enters is easily known, as ten litres are washed in each experiment. The importance of this arrangement is, as Cohn points out, that the germs develope, and the various species can be named macro-

scopically, while with the ordinary methods of examination of air it is impossible to do this, as the spores or germs are only seen, as such, and that by the aid of the microscope.

As precautionary measures, all the glass vessels used must be boiled for a long time in the water bath; corks are to be avoided, as they shelter spores in their cracks, and glass stoppers or gutta percha used in their place; and, lastly, the connection of the vessels which contain the nutrient solution must be so arranged, that no entrance of spores is possible after they have been boiled.

The passage of the air bubbles was regulated by a clip placed on the tubing, so that the ten litres of air took one to two hours in passing over. Notwithstanding this precaution an uncertain quantity of spores passed, apparently carried by the air bubbles, through the washbottles. After a certain quantity of air had been "sucked through," a precipitate or a troubling of the contained water was observed in these washbottles; white clouds of mycelium were, in all cases, observed on their walls and bottoms, which grew rapidly, and were easily seen by the naked eye; while on the surface of the nutrient fluid the mycelia fructified, and often allowed Cohn to see their hyphæ bearing the conidia. To hasten development he was in the habit of placing the washbottles, after a time, in his warm chamber (described Heft II, p. 196) at a temperature of 30° C. In one bottle he placed Pasteur's solution (with sugar), and in the other the normal Bacteria solution (without sugar). He always found *Aspergillus* and *Penicillium*; which he was able to distinguish from each other, by the loose character of the mycelium of the former. *Mucor* was found only once. On the natural assumption that each growth developed from one spore, Cohn finds that one such, capable of development, was obtained in every ten litres of air; and concludes from this that a man takes in 1000 such every day, of which, of course, the greater number are again passed out in expiration, or are in some way prevented from developing.

The cloudy appearance of the fluids was largely due to circular Torula cells, united in couples; the oval smaller form was rare; in the precipitate were found smut and other fungus-spores. "*As a rule no Bacteria were developed in the fluids of the washbottles;*" because, as Cohn thinks, they were with difficulty held back by the water, and rather passed over in the air bubbles without being wetted, as is the case with the spores of Lycopodium (Heft II, p. 189); he supposes, in effect, that they can only develope when thoroughly wetted through,—a condition which does not easily obtain,

unless they fall on a dirty surface, and are wetted there; as Cohn points out, these observations agree with those of Burdon Sanderson, although he is convinced that, when Bacteria did appear in the aspirator-bottles, they were really brought in by the bubbles of air, and washed out from them, and that their presence was not to be ascribed to any chance contact of surfaces.

3. Imbedded in a fluid which also contained *Micrococcus crepusculum*, Cohn found aggregations of varying size more or less circular in form, which, with their surrounding membrane, closely resembled frog's ova; one or more new aggregations were found in one membrane, the smaller circular, the larger irregularly elliptical; the latter formed a "cartilaginous" corpuscle, which was with great difficulty broken by pressure, not dissolved by ammonia, or coloured by iodine; but within them he found an aggregation of much smaller bodies of circular form, capable of being stained yellow with iodine, which were united together by a remarkably firm intercellular substance, and surrounded by a very thick cartilaginous envelope. These fresh characters are those of the genus *Ascococcus*. The cell-families thus formed break up into "nests" of irregular form, which are further divided into lobes, which are again subdivided into lobules, giving, in this way, to the whole the appearance of a bunch of grapes. Their development takes place in the following manner: small families of Micrococci are pushed towards the periphery of the surrounding gelatinous membrane, increase enormously, and thus get closely applied to one another; the gelatinous membrane grows at the same time; branches then appear, and the whole takes on a knobbed or iregularly bunch-like appearance; the larger lobes are, by further division, broken up into smaller ones, the membrane still embracing the whole family; but this separates the lobes when they grow too large, and produces forms of the appearance represented in figs. 4 and 5; and thus at last there are produced families of *Ascococci*, which, at first small and circular, become gradually larger, till they reach the stage represented in fig. 3.[1]

The chemical activity of this form is very remarkable; thus, it gives rise to a peculiar milky or cheesy odour, arising, as Cohn thinks, from the production of lactic acid, and lactic ether from ammonium tartate; and, further, the originally acid reaction of the nutrient solution is changed

[1] These numbers refer to Cohn's figures. Fig. 1 of our plate gives a good idea of the general appearance.

into one intensely alkaline, due apparently to the presence of free ammonia, which is very easily recognisable.

Although Cohn is not quite certain as to whether this form is really the same as that which Billroth has already called by this name, he thinks it probable that it is so; in any case he thinks his form one of generic value, and adds the following diagnosis of its characters:

Ascococcus, Billroth, char. emend. Cellulæ achromaticæ minimæ globosæ densissime consociatæ in familias tuberculosas globosas vel ovales irregulariter lobatas, lobis in lobulos minores sectis, capsula globosa vel ovali gelatinosa cartilaginea crassissima circumdatas, in membranam mollem facile secedentem floccosam aggregatas.

A. Billrothii, *n. sp., familiæ tuberculosæ* 20—160 *mikrom. capsula ad* 15 *mikrom. crassæ. In solutione ammonii tartarici acidi aëre lavata sponte ortum, membranam odore lactico vel butyrico præditam formantem observavi Mart.* 1874. *Haud scio utrum eandem an affinem speciem ill. Billroth in aqua carnis fœtida detexerit.*

4. Although, at first sight, the developmental history of *Ascococcus* is so remarkably strange, it appears to fill up a blank in the relations of the genus *Micrococcus* to the family of the *Chroococcaceæ;* for, in this family, there are forms either spherical or cylindrical, solitary or loosely connected, or forming distinctly limited colonies united by membranous intercellular substance; the genera *Glœothece, Microcystis, Polycoccus,* and *Anacystis,* for example, make solid balls formed of very small round cells, united by intercellular substance, and surrounded by an envelope of varying thickness; in *Polycystis* there are found several *Microcystis* colonies, with a common investment; while in *Cœlosphærium* the cells are found only at the periphery of the membrane, and so form a hollow sphere. Now, while *Ascococcus* is separable from *Microcystis, Anacystis,* and *Polycystis,* by the absence of colour in its cells, the developmental history of *Cœlosphærium,* as related by Naegeli, Unger, and specially Leitgeb, is strikingly analogous to that of *Ascococcus;*[1] *C. Naegelianum,* Unger, is a free-swimming form found on the surface of a pond at Graz, the cell-families of which form hollow spheres; these may unite to form 2—6 spheres with flattened edges; with furrows, more or less deep, and more numerous as the families are larger, on the surface; cell gradations between families with altogether indistinct fur-

[1] Cohn gives the following references: *Unger,* 'Denkschriften der K. Akad. der Wiss.' Band. vii. *Leitgeb,* Mittheil. der naturwiss. Vereins für Steiermark. Bd. ii, Heft 1, 1869. Tab. II.

rows, and united families are to be found; these latter may be broken up by pressure on the cover glass, but they also separate of themselves, and thus increase in number; in addition to this mode of propagation, which is the more common, single cells may leave the colony, and by repeated divisions form a new family.

The genus *Clathrocystis*, the cell-families of which are at first solid, and, later on, form hollow spheres of a beautiful pale green colour, and from 0,024—0.5 mm. diameter, presents a still more remarkable mode of division; which was first described by Henfrey,[1] in 1856, who saw numerous gonidia appear on the surface, in a single layer, imbedded in a colourless matrix; gradually the hollow sphere takes on the appearance of an irregular net, by the enlargement of fissures, arising at the periphery; the whole breaks up into fragments, which grow again into hollow spheres.

5, 6. Cohn now goes on to describe a new species of this genus, to which he has given the name *Clathrocystis roseo-persicina*, which he has found on leaves and like matter decaying at the bottom of glass vessels, as spots of a bright peach-bloom-red colour; under the microscope they appear as loosely aggregated cells, rounded or oval, which are either homogeneous or appear to be hollow, in which case the cavity is filled with one or more dark granules. Care must be taken not to confound with them other microscopic organisms, which have no real genetic connection with them, although of a similar colour. The first supply which led to the investigation of this form was received from Dr. Kirchner, who assisted Cohn in his labours; his specimens were found in a pond near Breslau, either on the surface of the water or mixed up with *Vaucheria*, *Spirogyra*, &c. Material was also received from Dr. Warming, of Copenhagen, and Mr. Ray Lankester, of Oxford. Much of the interest, indeed, taken in this plant is due to the investigations of Professor Lankester, published in this Journal, in October 1873;[2] this paper will be known to all our readers. The specimens found growing on other plants were of irregular form, but with a sharply marked contour, and consisted of cells united by a common gelatinous membrane; the free-swimming forms were hollow spheres, of a diameter not exceeding 0·6 mm. The contents were clear and fluid; and often the spheres exhibited cracks, with similar spheres and hemispheres arising from them; the

[1] 'Quart. Journ. Microsc. Science,' 1856.

[2] 'Quart. Journ. Mic. Sci.,' vol. xiii., N. S., p. 408. Pl. xxii, xxiii, and again vol. xvi, p. 27.

single cells were very small, only 2·5 microm.,[1] at times, in diameter, of circular, oval, or—owing to pressure—angular shape. The shades of colour, exhibited by their contents, always gave the Bacterio-purpurin absorption bands (Lankester), and exhibited the same chemical characters as that colouring matter, which is quite different to that of *Monas* (*Micrococcus*) *prodigiosa*. The cell-membrane, which resembles that of Glœocapsa, is generally very distinct, on account of this coloration; within are to be found very remarkable, dark granules; on account of their intercellular substance, which ordinarily separates the cells from one another by about their own diameter, Cohn would place this alga among the *Chroococcaceæ;* in addition to this, there is a general membrane for the cell-family, which is, however, observed with difficulty, unless some such pigment as carmine or gamboge be added to the water. The observation of their development is not altogether easy; at first the cell-families have not a circular but a vesicular or sack-like form; often, by the excessive development of cells in a certain direction, the whole takes on a bell-shaped form; these gradually become spherical, and further increase by the appearance of tertiary protuberances. If this *Clathrocystis* suffers from a want of water, daughter-cells are not formed, and the cell-families come to resemble Hydrodictyon by the appearance of large spaces in their midst; breaking up into smaller portions, they sink to the bottom of the vessel, and find a resting-place, on the decaying animal and vegetable matter which may happen to be there; from these, in time, spring up well-formed circular, and even net-like families of cells (fig. 10).

A remarkable mode of reproduction still remains: Cohn discovered young cell-families containing a definite (16—64) number of cells, united into a regular sphere, in such a way that they were closely pressed to one another, and seemed to fill the sphere; they differed from the rosy-peach-coloured families by an intensely purple-red coloration; but still more, by exhibiting spontaneous movement, turning round and round like *Volvox* or *Pandorina;* of its cause and of the length of its duration nothing can be said, but no such movement has ever yet been observed in any of the allied *Chroococcaceæ*.

7. *Monas vinosa* (fig. 13), Ehr. Next in order our author deals with the actively mobile, red corpuscles, which go under this name, and which he found in the same vessel with the peach-coloured *Clathrocystis;* of regularly spherical or oval form, often found in couples, their greatest diameter was 2·5

[1] 2·5 Micromillimeters = 0.0025 mm.

mikrom.; nothing could be seen of them save a pale red substance, in which were embedded darker granules; even cilia could not be seen. Their swarming movement was quite distinct from the ordinary trembling molecular movement, and it was due to it that they were enabled to collect themselves in groups on that side of the vessel which was turned to the light. Cohn thinks this form to be identical with that described by Ehrenberg in his 'Infusions-thierchen,' p. 11, in 1838. Charles Morren observed it, and gave an account of it to the Academy at Brussels in 1841, as forming the wine-red palmelloid crusts, which often extend to several inches in breadth, over water in which *Pteris aquilina* is decaying.[1] Perty's form *Chromatium* (*Monas*) *violescens*, Cohn thinks is probably the same as that described by himself as *M. vinosa*.[2]

8. *Monas Okenii* (fig. 12). Specimens of this form, which were also made known to science by Ehrenberg, were obtained through Dr. Hirsch, of Kahla in Thuringia, from a very rich pond in that place, the water of which was coloured red; the cause of this was the presence of a short cylindrical organism, ordinarily twice or three times as long as it was broad, with a diameter of ·005 mm., and a long axis varying from ·0075 to ·015 mm., according to the state of division in which it was found, with the ends rounded and generally slightly bent. It was found in enormous quantities, turning slowly on its axis; with this period of activity there alternated one of rest; they were mostly to be found on the side of the glass which was turned to the light; at first no organ of movement was seen, but the addition of iodine solution brought out a very long flagellum of more than twice the length of the body, behind which it was carried straight, or bent spirally. Just as in his *Clathrocystis*, Cohn found a varying number of dark granules in the interior of the organism; if one be dried, and alcohol added, the granules become coloured, and leave the protoplasm colourless, while one or two vacuoles appear in the limiting membrane. Acetic acid colours them a bright red; ammonia dissolves them, and gives a brownish-red colour to the pigment. These red corpuscles are the chief nourishment of the various kinds of animals, found in the water which they inhabit; curiously enough, their coloration in the Rotifera gives us some idea of the characters of the secretions of these animals; in the true stomach the corpuscles were of a bright red, due, as Cohn thinks, to the acid reaction of the gastric juice; while in the other portions of the intestine they were of a dark or brown-

[1] Mém. de l'Acad. de Bruxelles, 1841, p. 70.
[2] Perty, Kleinste Lebensformen, 1852, p. 174.

red colour, which was due to its neutral or alkaline reaction. They appear to propagate by fission.

Cohn now describes two new species, the leading characters of which must be rapidly enumerated:

9. *Rhabdomonas rosea*, n. sp. This form, which also came from Kahla, had a spindle-shaped appearance, with pale rosy-red corpuscles within, of ·0038—·005 mm, and a length ·02—·03 mm.; the cells were single and the corpuscles were dark, and highly refractive, in addition to which vacuoles were observed at the middle and ends; their movement was a trembling backwards and forwards, with a continual turning around their long axis; he has no doubt of the existence of a flagellum, though he only once saw it with distinctness.

10. *Monas Warmingii*, n. sp., was obtained from Dr. Warming, of Copenhagen, who found it in pools of brackish water, where marine plants were decaying, in the company of nearly all the forms—except *Ascococcus*—which have been described above; it resembled, save that it was broader, *Monas Okenii*; the body was clear, and the contained protoplasm firm and of a pale red, filled with dark red granules, *only at the rounded extremities*; length ·015—·02 mm., breadth ·008 mm.; their movement was irregular (taumelnd), but much more active than that of *M. Okenii*; posteriorly, there projected a flagellum; when division of the cells is about to occur, the middle, which was before devoid of granules, becomes filled with them, so that when division is accomplished the two new cells have the characteristic terminal groups of granules, at once; this form also differs from *M. vinosa* of Ehrenberg in its larger size.

11. *Ophidomonas sanguinea*, Ehr., a form of varying size and breadth, which Cohn found in Dr. Warming's water, is thought by him, after a long consideration, to be the same as that discovered by Ehrenberg in 1836. Although, indeed, since Cohn has himself shown, that *Spirillum volutans* has a fine flagellum at each end,[1] there is no difference between *Spirillum* and *Ophidomonas*; unless the smaller species (*S. termo*, *S. undula*) be shown not to possess flagella, when the generic term *Spirillum* would be justly theirs.

12. But a more important question is the relation of the Bacteria to the Monads; on the one hand, it may be said that the Monads are Bacteria. The Monad form here called *Clathrocystis roseo-persicina* was considered by Prof. Ray Lankester as a "peach-coloured Bacterium;" and Ehrenberg supposes that all the Bacteria possess cilia, (Frau Lüders, as

[1] 'Quart. Journ. Mic. Sci.,' April, 1873.

stated in the account of Cohn's earlier paper, has asserted the occurrence of one flagellum in B. termo[1]); and if this be the case, the Monads here described differ from them merely in their size. But on the other hand, certain Bacteria have very evident relationship with the *Oscillariæ* and *Spirulinæ*, which certainly do not move by the aid of flagella.

13. The highly refractive granules, already noticed in so many forms, and which are to be found even in the uncoloured Bacteria, are best known and most interesting in the genus *Beggiatoa*, specimens of which are to be found in every sulphur spring, as well as foul ditches, or other pieces of water containing a large quantity of organic matter.

14. This last Alga seems, indeed, to be the cause of the production of sulphuretted hydrogen, in the water in which it is found.

15. The two following sections on the separation of sulphur are of the very highest interest. On the fertile water received from Dr. Warming, Cohn observed a chalky scum, which was seen, by the aid of the microscope, to consist of small highly refracting granules, which did not effervesce on the addition of dilute acids; thinking, fairly enough, that this was due to the formation of sulphates, he applied other tests, which convinced him that he had to do with pure sulphur.

The formation of sulphur evidently depends on (1) the presence of a large quantity of sulphates in the water; and (2) on the absence of iron salts, which would cause the formation of ferrous sulphide. Now arises the question, is the formation of free sulphuretted hydrogen due to decaying or to living organisms? Although unable fully to resolve the question Cohn thinks the living organisms are the chief agents in the matter; for, in the first place, three species of *Beggiatoa*, one of which (*B. alba*) is found in hot sulphur springs, have been observed in such water, and the red-coloured organisms, already spoken of, seem to be not innocent; for they, like *Beggiatoa*, appear to increase under such conditions of impurity as would be fatal to all other organisms.

And, further, Morren, Fontan and Joly, and Menighini, have observed red or peach-coloured forms in sulphur springs. If this supposition, that such organisms can sustain life, in water full of free sulphuretted hydrogen, and consequently greatly wanting in free oxygen, be correct, we have to do

[1] *loc. cit.*, p. 158.

[2] Meyer: 'Journal für Prakt. Chemie,' xci, 1, Hedwigia, 1865. p. 81, &c.

with a most remarkable phenomenon; from which it may be very justly concluded, without fear of appearing hasty in drawing conclusions, that one form of activity in the circle of vital processes which certain organisms exhibit, is the production of free sulphuretted hydrogen, under suitable conditions.[1]

16. The curious highly refracting granules noticed so often already in the red-coloured organisms are similar to those which have been observed in *Beggiatoa;* they are, as Cohn shows, particles of sulphur.

By heating Beggiatoa-fibres on a slide the granules are caused to melt and form a large yellow drop, which gives off the smell of sulphurous acid; this experiment leaves no doubt as to their being sulphur; with polarised light they are doubly refractive, but their small size and high refractive index make it impossible for us to see their crystalline form. Similar experiments have succeeded with *Clathrocystis*, *Monas*, and *Ophidomonas*.

The question asked just above is now fully answered in favour of the living organisms.

17. The suggestion that *Bacterio-purpurin* is due to the formation of sulphur commends itself to Cohn, although he is unable to prove the truth of it, as yet. Mr. Lankester has pointed out that this colouring matter differs from that found by Schröter in *Micrococcus prodigiosus*, and Cohn points out that both differ, in the absence of chlorophyll, from the colouring matter of *Palmella cruenta* and other plants, such as the *Phycochromaceæ*.

18. Blood-coloured milk was observed to owe its appearance to the colouring matter of *M. prodigiosus*, which Cohn, by the way, finds to be soluble in alcohol and other, unlike Bacterio-purpurin, as well as in the fat-globules (Buttertropfchen) of milk. An interesting use of the microspectroscope is also noted; Cohn recognised the colouring matter of *M. prodigiosus* in a capillary vessel containing the red milk, where he was unable to recognise by the microscope the forms of the organism which produced it.

19. Our author remains true to his original position, that the Bacteria may be arranged in two series; one containing *Micrococcus* and *Bacterium*, which run parallel to the *Chroococcaceæ*, and pass through a resting or *zooglœa* stage, while the other contains *Bacillus*, *Vibrio*, and *Spirillum*, which run parallel to the *Oscillariæ*, and pass through the *Leptothrix*, as their resting stage; and this, although many of the *Oscillariæ* become surrounded by gelatinous membrane, that is,

[1] The Euglenæ also exist and increase in such waters.

take on the appearance of zooglæa; and while Professor Lankester went a long way towards convincing him that *Spirillum undula* formed zooglœa masses (cf. 'Quar. Jour. Micro. Sci.,' xiii, Plate XXII, figs. 8 and 9), Cohn himself saw a colourless Leptothrix-like fibre in a "glia"; but as he never observed genetic connection between this form and *Spirillum*, he thinks it better to call it *Myconostoc* n. g., as it is among the Bacteria, a form parallel to Nostoc among the Algæ, and to give it the specific name of *gregarium*, because of its social character.

20. Another new genus (*Cladothrix dichotoma*), which Cohn has lately observed, gave him, at first, a great deal of trouble; he found colourless fibres very finely granulated, and apparently unsegmented, which resembled *Leptothrix*, save in this that they appeared to branch with great regularity; this was difficult to believe, because neither *Leptothrix* nor *Oscillariæ* ever present such branchings; but examined with a higher power, these organisms were seen not to present a true dichotomy, but that false branching which is so characteristic of the *Scytonemeæ* allied to the Oscillariæ, which is brought about by the apposition of two fibres, which, while growing divergently at their upper, grow in apposition at their lower ends.

This mode of origin of false branches agrees so closely with that observed in *Schizosiphon* and other *Oscillariæ*, that Cohn congratulates himself on the discovery, as tending to confirm his views of the close relationship which these putrefactive forms have to the *Oscillariæ*.

21. The next Bacterium described is also a new species, although the genus *Streptothrix*—the new form has the specific name *Foersteri*—is well known; the place in which this form was found was the lachrymal canal, where such organisms were first observed, in 1855, by the famous von Graefe, since which time other observers have also noticed them. Professor Foerster coming across it sent specimens to Cohn, who recognised specific differences between this form and th- *L. buccalis* which is found in the mouth, inasmuch as this latter is thicker, straight, while *L. Foersteri* is screw-shaped, like *Spirochæte*. *L. buccalis* is never branched as the new form frequently is. The full account of this form and the elaborate comparison which he sets forth between it and *L. buccalis* will have much interest for those who busy themselves with these remarkable parasites.

22. It would be strange, indeed, if any writer on Bact in the sad times in which our lot is cast, should not hav say about the question of spontaneous generation, fl

although we have been with literature on the subject. Cohn's discussion on "lasting spores" (Dauersporen) is really of interest. He has already referred to the subject in his previous essay; there noting the great probability of *Bacillus* being reproduced by gonidia or lasting spores, which had an oily highly refractive internal substance; three observers, Perty, who called them *Hygrocrocis*, Trécul, who described them as *Urobacter*, and Billroth, who gave them the name of *Helobacteria*, had already observed these spores. Cohn having carefully repeated Bastian's experiments,[1] found that after three or four days the fluid was troubled, not by *B. termo*, but by *Bacillus*. Wondering, therefore, whether there were not certain forms of these organisms which could withstand the effects of boiling, he applied himself to what we will call the natural history of cheese.

23. Having observed the whole process, he thinks the operation consists of three parts:

(1.) The coagulation of the milk, which, as he says, is due not to organised, but merely to non-organised ferments (chymosin), comparable in action to diastase and pepsin.

(2.) The separation of the coagulated casein from the whey, which is a purely mechanical process, comparable to the separation of butter from milk, or fibrin from blood.

(3.) The coming to maturity of the cheese, which is a true fermentation, caused by ferment organisms (Zymophytes), accompanied by the evolution of gases and the formation of spaces, as in bread; the heat which is applied (55—60°) kills *B. termo*, and appears to leave free play for the activity of the peculiar ferment organisms of the cheese.

24. The Bacilli, represented in figs. 6—8, were more closely studied in the laboratory at Breslau; they were obtained from the rennet of a calf, of five to seven weeks old. It was found that their activity was lessened at 50° C., but below 30° was destroyed; they were seen to be actively moving, long, fine Bacilli, ordinarily paired, but sometimes united by fours or eights, never absent from the extract of the rennet. They are probably found in the living stomach, and such forms have, indeed, been already observed by Remak (1845) and Wedl (1858); they also appear to be the same as Pasteur's *B. subtilis*, which are so remarkable for their resistance to high temperatures, as well as for their activity in air deprived of its oxygen. If the fluid in which these are found be kept for some time at 30°, there are to be observed fibres with a swollen extremity, filled with oval slightly refractive granules, which, closely resemble in form the

[1] 'Proc. Royal Soc.,' 1873, No. 145.

spermatozoa of vertebrates, and rapidly increase in number; these are clearly either Heterocysts, or, as is more probable, *spores*, which belong to the life-history of *Bacillus*, and are produced under certain conditions. If this be so, Bastian's experiments are explained, for the spores were able to resist the comparatively short boiling to which they were subjected, and easily grew to maturity in the nutritive fluid in which they found themselves. In many other cases Cohn says he has observed such lasting spores; and lest some one, tempted by peas instead of cheese, should feel inclined to write two volumes on the 'Beginnings of Life,' it had better at once be added, that a similar putrefaction was observed in a solution of peas in *distilled water, which had been kept for several days at* 45° C.

25. *Spirochæte Obermieri* is already known to English pathologists from Dr. Burdon Sanderson's Report on the Pathology of Infective Processes,[1] where he figures, from a drawing sent by Cohn, the "spirillum" of relapsing fever. So full an account is there given, that it will be here sufficient to add what Cohn has since discovered concerning these organisms. They do not belong to *Spirillum*, but to the genus *Spirochæte*, of which only one species, *S. plicatilis*, has been hitherto known; and that, thanks to the labours of Ehrenberg, who, like Cohn, found it in the water of marshes. This latter differs from *Spirillum* in the character of its movements, which are at times serpentine; the screw-like windings of the fibres are constant in character, but their length is most variable. In addition to the serpentine movements, by which they are enabled to change their place, they also exhibit an undulation passing along their whole length, which has never been observed in *Spirillum*. Cohn is unable to say whether the form found in the blood is the same as that which is found in marshy water; if so, it is quite possible that the fever was produced by drinking it; another Spirochæte, however, was once observed by Cohn in the mucus of his own mouth, which appears to be intermediate between these other two forms.

It may be added that osmic acid, as Weigert has shown, preserves their characters unchanged.

26. The [bacterium of splenic fever was also cited by Dr. Burdon Sanderson;[2] Cohn now gives it the name of *Bacillus Anthracis*, which differs from the *Bacillus* of butyric fermentation (*B. subtilis*), in being shorter and firmer, and

[1] Report of Medical Officer of the Privy Council, &c., N. S., No. III, 1874, p. 41.

[2] *loc. cit.*, p. 35.

never exhibiting movement; he cannot agree with Bollinger in thinking that the rods are formed by rosary-chains, and says that he has only found the refractive spheroids in dead specimens; while the question of their relation to Micrococcus must still remain undecided. Further, this form appears to agree with other *Bacilli*, in the possession of lasting spores, by which probably the contagium is spread. Brauell has shown that the placenta forms a true filter, and that the blood of the fœtus may be free from *Bacilli*, while that of the mother is full of them; and as the fœtus does not suffer from splenic fever, Bollinger is justified in asserting that the organisms themselves, and not the blood, are the cause of offence.

27. Cohn just refers to the Bacterium of the diseased silkworm, which he has called *Micrococcus bombycis*, oval bodies, something like *M. ureæ*, arranged either singly or in chains of varying length; he hopes to go into their history in the next issue of his 'Beiträge.'

28. In conclusion, there are a few remarks on the natural relationships of the genera of Bacteria, with a classification, which is appended in full; the position which Cohn has taken up, and which is now generally accepted—that is, that the Bacteria are plants, more closely allied to the Algæ than to the Fungi—is again supported by our knowledge of the forms just described; consequently neither Naëgeli's name *Schizomycetes*, nor Cohn's *Schizosporeæ*, are very good; perhaps *Schizophytæ* is the most suitable name for this first and simplest division of living beings, even if its characters are more negative than positive. The cells of the *Schizophytæ* are either free, or united into cell-families; in the latter case they are arranged either in one plane or on the surface of a sphere, or as cellular bodies; the groups thus produced may be formless, or surrounded by a definite and common envelope; the cell-series may be simple, or exhibit spurious branchings, and these series may be single or united into bundles. They increase by binary cell-division, and the divided cells may be all in one plane, or arranged as a cross, or in all three directions. Sexual reproduction is unknown; lasting spores have been frequently observed; spontaneous movements have been seen, at least for a short time; they cannot be separated, save for convenience, according to their colours and size; the cells are various in size and shape.

Schizophytæ.

Tribe I.—*Glœogenæ.*

Cells free, or united by intercellular substance into cell-families.

A.—Cells free, or united in twos or fours:
- Cells rounded . . . . . *Chroococcus*, Naeg.
- Cells cylindrical . . . . . *Synechococcus*, Naeg.

B.—Cells united into an amorphous gelatinous family in their resting stage.

a.—The cell-membranes confluent with the intercellular substance.

I.—Cells very small, and without phycochrom:
- Cells rounded . . . . . *Micrococcus*, Hall. emend.
- Cells cylindrical . . . . . *Bacterium*, Duj.

II.—Cells larger, with phycochrom:
- Cells rounded . . . . . *Aphanocapsa*, Naeg.
- Cells cylindrical . . . . . *Aphanothece*, Naeg.

β.—Intercellular substance formed by the cell-membranes running into one another.
- Cells rounded . . . . . *Glœocapsa*, Kg. Naeg.
- Cells cylindrical . . . . . *Glœothece*, Naeg.

C.—Cells united into a gelatinous family, of well-defined form.

a.—Cell-families in one layer:

I.—Cells arranged in fours, in one plane, *Merismopedia*, Meyen.

II.—Cells in no order, arranged on the surface of a sphere.
- Cells rounded; the families form nets by fission . . . . } *Clathrocystis*, Henfr.
- Cells cylindrical and wedge-shaped; the families divide by the appearance of furrows } *Cœlosphærium*, Naeg.

β.—Cell-families in several layers, united into spheroidal cell-bodies:

I.—Cells definite in number.
- Cells rounded; colourless; in fours . . . . . } *Sarcina*, Goods.
- Cells cylindrical and wedge-shaped, in no order, with phycochrom . . . . . } *Gomphosphæria*, Kg.

II.—Cells indefinite in number, but that very large.
- Cells colourless, very small . . *Ascococcus*, Billr. emend.
- Cells larger, with phycochrom . { *Polycystis*, Kg. / *Coccochloris*, Spr. / *Polycoccus*, Kg. et al.

Tribe II.—*Nematogenæ Rab.*

Cells arranged in rows.

a.—Cell-threads free, or in a felted mass:

I.—Threads cylindrical, colourless, indistinctly jointed.

Very fine and short . . . . *Bacillus*, Cohn.
Very fine and long . . . . . *Leptothrix*, Kg. em.
Thicker and long . . . . . *Beggiatoa*, Trev.

II.—Thread cylindrical, containing phycochrom, distinctly jointed:

Reproductive-cells unknown . { *Hypheothrix*, Kg. / *Oscillaria*, Bosc. et al.

III.—Threads cylindrical, jointed, forming Gonidia:

Colourless . . . . . *Crenothrix*, Cohn.
With phycochrom . . . . . *Chamaesiphon*, et al.

IV.—Threads screw-shaped:

1. Without Phycochrom.

Short, slightly undulating . . *Vibrio*, Ehr. em.
Short, spiral, stiff . . . . . *Spirillum*, Ehr.
Long, spiral, flexible . . . . *Spirochæte*, Ehr.

2. With Phycochrom.

Long, spiral, flexible . . . . *Spirulina*, Link.

V.—Threads form a rosary-chain:

Without phycochrom . . . . *Streptococcus*, Billr.
With phycochrom . . . . { *Anabæna*, Borg. / *Spermosira*, Kg. et al.

VI.—Threads whip-like, growing at the point . . . . . } *Mastigothrix*, et al.

β.—Threads united into gelatinous families by their intercellular substance:

I.—Cylindrical and colourless . . . *Myconostoc*, Cohn.
II.—Cylindrical, with phycochrom . { *Chthonoblastus*. / *Limnochlide*, Kg. et al.
III.—Forming rosary-chains . . . *Nostoc-Hormosiphon*, et al.
IV.—Whip-like, growing at their point { *Rivularia*, Roth. / *Zonotrichia*, Kg. et al.

γ.—Threads exhibit spurious branching:

I.—Cylindrical and colourless . . { *Cladothrix*, Cohn. / *Streptothrix?*
II.—Cylindrical, with phycochrom { *Calothrix*, Kg. / *Scytonema*, Kg. et al.
III.—Forming rosary-chains . . { *Merizomyria*, Kg. / *Mastigocladus*, Cohn.
IV.—Whip-like, growing at their point } *Schizosiphon*, Kg. / *Geocyclus*, Kg. et al

In the paper which concludes this 3rd Heft, Dr. Edward Eidam, who has been working under Cohn's direction in the University of Breslau, takes up the interesting questions of the influence of different temperatures, and of drying on *Bacterium termo*, Duj., with special regard to its development. Cohn himself had worked at the subject, and in his previous paper (in Heft II) had found the maximum mortal temperature (the experiment lasting for an hour) to be about 60° to 62° C. But both Cohn's points and that of Schröter

(59° C.) apply only to Bacteria *in a clear watery fluid.* On the other hand, Horwarth has studied the minimum mortal temperature, and finds that Bacteria can survive after having been exposed to a temperature of —18° C.; at such a point, indeed, they pass into cold rigor, but at a higher temperature again become vitally active. The breeding and feeding solution used was that which Cohn has already described; but, as in the previous account in this Journal, the proportions of the constituents were not given, it may be well now to add them:

Potassium phosphate . . .	1·0
Magnesium Sulphate . . .	1·0
Ammonium Tartrate . . .	2·0
Calcium Chloride . . .	0·1
Distilled Water . . .	200·0

If kept at 50° C., this solution will remain quite free from Bacteria.

From eighteen experiments carried out for the purpose of seeing the effects of different temperatures, Eidam concludes that at 5° C. there is no reproduction (here it should be remarked, that a similar tube to those which produced this result was placed in a warm chamber at 30° to 35° C., and that Bacteria were actively reproduced in it); but at 5½° C. reproduction began, though very slowly, and so on up to 10° C.; above which point reproduction began to be much more energetic, and so continued up to 30° to 35° C.; above 35° C. it fell, at 40° the Bacteria fell into heat-rigor, and died at 60°.

From these experiments also could be drawn a confirmation of what Cohn in his first paper so wisely remarked, "Putrefaction is a correlate phænomenon, not of death, but of life;"[1] for in those tubes, to which the drop containing Bacteria was added, without an increase of these organisms becoming apparent—that is, in those kept at very low temperatures—there was no putrefaction, but as the temperature was raised, and the Bacteria began to multiply, this concomitant of Bacterian vitality began to show itself, and with these conditions the intensity of the smell of putrefaction went hand in hand.

But there was a smell, as of cheese, in some tubes kept at 44° to 46°, to which albumen or peas had been added; whence came this so sure, even if so disagreeable sign of vitality, if it be true that at such temperatures *Bacterium termo* is in a state of heat-rigor? Microscopical examination revealed the presence of Bacillus-filaments and lasting spores;

[1] Heft II, p. 204.

and such observation was sufficient to satisfy the author, that butyric fermentation was taking place.

The experiments on the influence of the length of the time of heating on Bacteria showed that—

(α) Heating for twenty-five hours at 40° was not sufficient to kill them; but the delay in commencing to increase in number which was observed when they were placed in the warm-chamber, showed that they had suffered from heat-rigor.

(β) Thirteen to fourteen hours' heating at a mean temperature of 46°, or

(γ) Three hours at 51° was sufficient to kill them.

But if *Bacterium termo* be exposed to a temperature of 50° C. in the *dry*, it still retains its vital activity even if the experiment be continued for as much as six hours, (lower temperatures such as 15° to 16° continued for a week did not destroy the Bacteria); such an experiment would tend to show that the surrounding membrane must be of considerable value, as a protector to the protoplasm within.

Finally, glass rods that had been dipped in a fluid containing Bacteria, and then dried for an hour at 15° C. were brought for a moment in contact with ammonia, alcohol, crude carbolic acid, and acetic acid; and again dried for an hour in the air; the only liquid that proved fatal was the last.

Eidam concludes by insisting on a careful and continuous study of the varying conditions of life, which obtain among the Bacteria.

Note *on* Bacterium rubescens *and* Clathrocystis roseo-persicina. By Professor Lankester, F.R.S.

I am continually examining new and varied growths of *Bacterium rubescens*, and the more I see of it, the more I am convinced that Professor Cohn's separation of it into the various species of Clathrocystis and Monas is altogether untenable. I have before me whilst writing this a rich growth which has the characteristic redder tint (not the bluer peach variety) of Bacterio-purpurin. This growth consists of a dense mass of red-coloured Vibrio of the typical form. I consider it to be like the red-coloured Spirillum described by Dr. Klein in this Journal, October, 1875, and the *Ophidomonas sanguinea* of Ehrenberg—a form-phase of *Bacterium rubescens*.

When I look at the feebly-magnified and really unsuccessful portraits which Professor Cohn has given of these red-coloured Schizophytes (some of which are copied here for the use of readers), I am less surprised at his maintaining the generic and even ordinal distinction which he assigns to them than I should have been, had his drawings given evidence that he had devoted to these forms that care which they deserve.

In the first place a higher power than 600 diameters should be used, and drawings of the individual cell of *every variety* of form and structure should be given in illustration of the view that Clathrocystis roseo-persicina is distinct from Monas vinosa and M. Okenii. Instead of this, I must most distinctly point out that Professor Cohn *has omitted to figure the intermediate forms* which are abundant in most gatherings, and were so in those which I sent to him. In fact, either my figures of such forms in my papers in this journal in 1873 and 1876 are inventions, or Professor Cohn has ignored their existence. It must be remembered that Professor Cohn has had the opportunity of examining two samples of these organisms sent by me, in support of the view which I have taken with regard to them.

Whilst referring the reader to my previous papers—the statements in which I can from new studies made this summer most fully confirm in every particular (excepting as to the movements of the large bacterioid plastids, which I did not discover until after my first paper was published)—I may here draw attention to some inaccuracies in Professor Cohn's descriptions of the Clathrocystis-forms, and the so-called Monas-forms of Bacterium rubescens.

Use of the generic term Monas.—When we inquire into the characters which both Ehrenberg and Dujardin have assigned to the family Monadina, as indicated by the forms placed there by them, we find that the most essential feature of a "Monad" is the possession of a remarkable vibratile cilium, which is extended *in front* of the organism, and which it *follows* (as it were) in its movements. This characteristic monad-cilium, which may be called a "præflagellum," must be broadly distinguished from a "postflagellum," such as the tail of a spermatozoon, which *drives* the "head" before it—the whole mechanism of the movement in the two cases is in contrast, and the two organs (the leading and the driving flagellum) deserve to be distinguished more widely than any two forms of vibratile protoplasm. *Euglena* and *Astasia* furnish admirable examples of the præflagellate form as well as the common *Monas lens*. The genus Bodo is

distinguished by being amphi-flagellate, that is provided with both præ- and post-flagellum; other Monadina are doubly præflagellate, none are simply post-flagellate.

Amongst the Schizophytæ Professor Cohn has described *Spirillum volutans* as being amphi-flagellate, whilst Messrs. Dallinger and Drysdale have made a similar discovery (using it is true the very highest powers of the microscope, and succeeding only with very great care and persistence) with regard to *Bacterium termo*.

I am not aware that the simple post-flagellate form has hitherto been recognised as characteristic of any monads, in fact the essential feature of a monad is that it is præflagellate. Yet Professor Cohn does not hesitate to leave the forms known as *Monas Okenii*, *M. vinosa*, &c. in the genus Monas after he has ascribed to them a single coarse post-flagellum. The possession of this postflagellum and the absence of a præflagellum seems to me to necessitate the separation of these forms from the Monadina whilst it is by no means inconsistent with the view that they are form-phases of a Bacterium.

The flagellum of the locomotive plastids of Bacterium rubescens.—Professor Cohn has figured (see the plate accompanying the abstract of his paper) postflagella of extraordinary coarseness as attached to the locomotive plastids of *B. rubescens*, which he speaks of as *Rhabdomonas rosea*, *Monas vinosa*, *M. Okenii*, and *M. Warmingii*. He appears not to have been able to see these flagella satisfactorily in all cases, which would be surprising were they as thick as he has represented them to be. I have most carefully examined a variety of locomotive plastids of *B. rubescens* corresponding to each of Cohn's three monad-species and other varieties which he does not mention. In none of them, not even the largest which attain a length greater than that of *Monas lens*, which is found with them and has an easily visible *præflagellum*, could I get sight of the flagellum, either by long watching in the living state or by the use of reagents such as iodine, magenta and osmic acid. Occasionally I have seen, after treatment with reagents, a coarse filament adherent to one of the locomotive plastids in question, but its presence seemed to me to be accounted for by the fact that small filamentous vibriones and spirilla were abundant in the liquid. At the same time I do not feel *any doubt* that these locomotive plastids possess one or more postflagella of excessive tenuity. Under a No. 10 immersion of Hartnack, one may follow for half an hour or more the movements of one of these active plastids. I have done this with such a large form as that figured by

me in this Journal, vol. xiii, pl. xxiii. fig. 12, and after tracking it here and there over the field, have seen it arrested by some obstacle, when a lashing movement can be most distinctly traced to a distance of thrice its own length behind the plastid. I have never seen any indication of such a lashing movement in *front* of the plastid. The presence of highly refracting globules (loculi) in these large plastids enables one most clearly to note the constant *rotation* of the plastid on its own long axis. This frequently continues with great activity for some seconds after locomotion has been arrested. It is worth noting that the direction of linear motion and of rotation is never permanently reversed in these forms.

In dividing specimens of Bacterium termo, I have seen the chain dart first in one direction, and then without turning round *return* on its path with equal velocity. This can only be due to the existence of a driving flagellum at *each end* of the dividing chain. In the motile plastids of Bact. rubescens I have *not* seen this active reverse movement but only a hesitating and feeble reverse movement, which soon gives way to renewed rapid movement in the original direction. Probably Bact. rubescens is, like B. termo, amphiflagellate, but has one (the younger formed at the pole of the last fission) of its flagella much smaller than the other. Neither of these flagella in any Schizophytæ appear to be 'leading,' but always driving flagella.

It does not appear to be necessary to have recourse to a postflagellum to account for the movements of the remarkable acicular plastids (figs. 28, 29, vol. xiii, pl. xxiii) which I have found again in great quantity and beauty in specimens of the crusts of *Bacterium rubescens* taken from the surface of dead ivy-leaves at the bottom of a rain-water tank in the Botanic Garden, Oxford. The slow serpentine 'crawl' of the leptothrix-filaments of *B. rubescens* which I have frequently witnessed, is similar to that of Oscillariæ and equally difficult to explain.

Dark granules supposed to be Sulphur by Professor Cohn. The dark granules described by Professor Cohn in the motile forms of Bacterium rubescens (his Monas vinosa, Okenii, &c.) and figured by him as black specks (see his figures), are not truly dark. What he has taken for *dark* granules are in reality very bright, clear, highly refringent globules, which I have spoken of in my previous papers as 'loculi.' When the loculi are small they appear under feeble magnifying power as black specks. These loculi appear to be a more active part of the plastid differentiated from the surrounding

substance which tends to take on the character of a cell wall. The loculi, as I have shown, may be minute or relatively large, few, numerous or absent; and all these varieties occur in one and the same *form* of plastid.

That they contain sulphur is possible, as Professor Cohn thinks he has shown, but that they consist of sulphur, cannot, I think, be admitted. They appear to me rather to be specially active points in the differentiating protopolasm of which the Bacterium-plastid consists.

The manner in which they are represented in Professor Cohn's figures, is not at all like their real appearance when examined by Hartnack's No. 10 immersion. This is more nearly given in my fig. 12, of pl. xxiii, vol. xiii of this Journal, and other figures on the same plate.

The shape and minute structure of the plastids which build up the Clathrocystis-like growths of Bacterium rubescens. Professor Cohn says that the form of the plastids in what he calls Clathrocystis roseo-persicina is spherical, oval or angular. His figures are for the most part not sufficiently magnified to give an idea of the real structure of the constituent plastids. I must assert in opposition to Cohn that the plastids in question are often elongated, and form 'mycoderma-like' and 'merismopedia-like' tessellate aggregates of oblong plastids. Further it is not true that the highly refringent globules (erroneously termed 'dunkle Körnchen') by Cohn, and discussed above, are embedded in the cell-contents. The plastid of this Clathrocystis-form may be perfectly homogeneous with no differentiation of cell-wall and cell-contents, or you may have (*a*) one, two, or more minute highly-coloured 'loculi' differentiated in the otherwise homogeneous plastid (these are Cohn's 'dunkle Körnchen'), or you may have (*b*), a great loculus highly coloured occupying the whole of the medullary part of the plastid which may then be said to consist of cell-wall (wall of the loculus) and cell-contents (coloured, highly refringent substance filling the loculus). These structural features are fully illustrated and explained in my first paper.

Hydrodictyon-like networks. The condition of the Clathrocystis-phase of Bacterium rubescens, which Professor Cohn compares to Hydrodictyon, is unfortunately chosen, for as will be seen by comparing his figure with fig. 19, pl. xxiii of vol. xiii. of this Journal—the condition which I have there figured and have elsewhere compared to Hydrodictyon both as to its actual form and mode of development—is very much more closely in agreement with the beautiful green net-alga. The figure which I have given represents only a small frag-

ment of a great basket-work of the same structure constituting a hollow globe, such as would have filled an entire plate if drawn to the scale adopted.

Omission of Leptothrix forms by Professor Cohn.—Very abundant in many growths of Bacterium rubescens are long Leptothrix filaments containing loculi coloured by Bacteriopurpurin. Of these and their significance Professor Cohn omits all mention. The fact that they exist and that between them and the biscuit-shaped or oblong plastids *there are completely transitional forms*, such as those figured in pl. iii, fig. 3, of this Journal, January 1876, is sufficient to upset once and for all the position taken up by Professor Cohn, to the effect that the Bacteriaceæ can be arranged in two natural series the one containing Micrococcus and Bacterium parallel to the Chroococcaceæ—the other containing Bacillus, Vibrio and Spirillum parallel to the Oscillariæ. According to Cohn the first series have their quiescent stage exclusively as glœogenous cell-families; whilst the other series have their quiescent stage exclusively as Leptothrix filaments.

The fact that *Bacterium rubescens* occurs both in the glœogenous (chroococcaceous) and the leptotrical (oscillarian) conditions renders this classification of Cohn's quite erroneous.

Moreover it is to be remarked that the active movement of leptothrix-forms and of Oscillariæ renders a balancing of them against the vegetative resting stage of Chroococcaceæ inappropriate.

Résumé *of* Recent Contributions *to our* Knowledge *of* "Freshwater Rhizopoda." Part I. Heliozoa: Compiled by Wm. Archer. (With Plate XXI.)

A large number of contributions to our knowledge of "Freshwater Rhizopoda" have recently made their appearance in Germany in the 'Archiv für Mikroskopische Anatomie,' formerly edited by Max Schultze, and as it is not unlikely that a careful and diligent out-look may reward observers in our own country with the discovery from time to time of some of the interesting new forms brought to light in these Memoirs, it has been thought that a *résumé* containing as complete an account as our space permits will be of interest to the readers of this Journal.

Of these recent memoirs the most important and complete is that of Hertwig and Lesser.[1] Those authors give a systematic arrangement of the forms, which, brief and preliminary as it may be and, even though it should turn out to be possibly only provisional, must, so far as our knowledge of the forms goes, serve in the meantime a useful purpose. This I purpose to recapitulate after giving a running description of the various new forms lately introduced by those and other (almost contemporary) observers.

Hertwig and Lesser regard the lowly organisms comprehended under the general name "Rhizopoda" as wanting in those definite characters which would connect them, on one or other side, either to the animal or vegetable kingdom, and hence they hold they must be relegated to the "Protista." It would be out of place here at all to attempt to discuss whether or not there be a real necessity for the formation of a kingdom so-called. Suffice it that the forms falling here are admittedly amongst the most lowly of organisms, few of which become elevated above the simple stage of a single "cell." These authors conceive that they (like colourless blood-corpuscles or wandering connective-tissue-cells) are but composed of an undifferentiated protoplasm, whose vital activity forms the common seat for all the functions.

I venture myself to suppose this may be a somewhat too general assertion, for the sharp line of demarcation to be seen in certain Heliozoa between endo- and ectosarc and the seeming restriction to, or at least chief retention of the incepted food in, one or other region (in different species with and without "skeleton") would seem to point to a certain amount of restriction of location of at least the digestive function, to a definite part of the body-substance.

On the whole, indeed, one must agree with the authors that nowhere can we say *absolutely* that this or that part (and *no other*) subserves to nutrition, to perception, to movement, to reproduction, but rather we are compelled to the assumption that one and the same portion which may, for instance, subserve to motion, may take a share likewise in assimilation and perception.

In these simple protoplasmic (sarcodic) beings the subject of consideration, motion and contractility, is a property of the whole body-parenchyma. This renders itself evident internally by a circulation of the granules embedded in the plasma and externally by a change of place and by the

[1] Drs. E. Hertwig and E. Lesser, "Ueber Rhizopoden und denselben nahestehende Organismen," in 'Schultze's Archiv f. Mikr. Anatomie,' Bd. x, Supplementheft (1874).

alteration of the body-contour. Locomotion is effected in two ways. In one case it takes place by contractility, affecting the whole body-substance in a uniform degree. By this mode the body alters in contour but little and glides along by virtue of a constantly rotating motion of its superficies over the surface of the objects on which it finds itself (*e. g.* Hyalodiscus). In other cases it is only restricted portions of the surface by the agency of whose contractility locomotion is effected, either by a flowing onwards of the body-substance or by the projection of sometimes pointed, sometimes blunt, pseudopodia, by aid of which the organism draws or pushes itself onwards. When a *test* is formed the place of origin of the pseudopodia becomes limited to definite parts of the body; if naked they may be given off from any part of the whole body-superficies. Under all circumstances the pseudopodia are simple prolongations of the body-substance, into which they can flow back just as they imperceptibly take origin from it. They are not only organs of locomotion, but, like the body-plasma, possess the faculty to digest foreign bodies—in some forms whose shell does not allow of the passage inwards of large objects as "food," not only their capture but their digestion is performed by the pseudopodia.

The food is taken bodily into the substance of the organism, and becomes embedded directly in the protoplasm. Any definite apparatus serving to the formation of any digestive fluid is quite absent. It is true that captured objects become embedded in a vacuole and surrounded by a fluid, but this cannot be regarded as a special digestive organ, since it originates and disappears with the coming and going of its contents. Any once-for-all preformed place, by possibility to be designated as a mouth, does not exist; but any exposed part of the superficies (body or pseudopodia) can incept foreign bodies. There does not seem except in rare cases any particular *choice* of food, though some forms are very, if not wholly, abstemious, whilst others are all but insatiable.

In many Amœbæ the differentiation into an outer homogeneous and an inner granular substance which exists has been held to indicate a differentiation into a contractile cortical, and a medullary region, the latter performing the digestive function, still the distinction is no decisive one, but is gradual, inasmuch as the transition of one substance into the other is imperceptible and there exist no definite sharp limits between them.

A whole series of organisms do not advance beyond the

condition of a little protoplasm-mass—remaining all through their existence " beings without organs "—these are Häckel's " Monera."

A still greater number, however, reach a higher development, producing in their interior a nucleus, with its nucleolus. This body where it occurs maintains a remarkable constancy of its form. A clear vesicle, whose contents coagulate only sparingly or not at all on the application of acetic acid (nucleus), encloses a body either of a simple oval figure or divided into several portions and of pale bluish tint, which, under dilute acetic acid, coagulates in a darkly-granular manner, in strong solutions, swelling up, without, however, becoming dissolved (nucleolus). The outermost zone of the nucleus frequently forms a membranous layer.

Hollow spaces (vacuoles) filled with fluid are very prevalent in the protoplasm ; they are present either in changeable number and in any position, or they are restricted to definite parts of the body and then limited in number. They often originate and disappear periodically—an alternation which proceeds, in the last-mentioned cases constantly, in the first frequently, with the appearance of rhythmical contractions. Under all circumstances these represent nothing more than vacuities in the protoplasm, filled with fluid, and they are quite destitute of any definite wall.

The "skeleton" (when present) is formed of a number of isolated spines and spicules of the most different form and arrangement, or of a single connected piece of varied form. A greater degree of firmness and resistance in the skeleton parts is arrived at by depositions of mineral constituents, chiefly of carbonate of lime or silex; more rarely foreign bodies, such as minute fragments of silex or of diatoms are cemented on the shell.

The same simplicity, characteristic of the group, shows itself also in the development—division only of the total body-mass serving to multiplication. Any special organ destined thereto is not present: at least the interpretation of the nucleus as a brood-cell for daughter-organisms, put forward by many, is not supported by any certain or connected observation. In their simple development-cycle encysting has a wide-spread place. The organism assumes a globular shape, and excretes a simple or multi-laminated coat. After some time the body breaks up into two or several portions, boring through the wall and each assuming the form of the mother-organism. The interpolation into the cycle of their life-history of the encysting process would seem justly to be considered as a condition only subsequently, as it were,

gaining a footing in the development-cycle of these simple organisms; it would seem as if this process may have at first originated in an adaptation to external circumstances—such as the drying up of the circumambient medium or after copious nourishment, in order to digest at leisure, fortified against surrounding foes—and that it became only afterwards turned to use as a reproductive process.

As to the limitation and affinities of the group here under consideration, the authors point out that amongst Protozoa the Gregarina and Ciliata stand the most remote, for the formation of trichocysts, muscle-striæ and a cuticle present the first beginnings of a true tissue-formation in unicellular organisms; in them locomotion and alterations of the figure of the body are performed by definite organs. In the Ciliata there exists a definite "mouth" (Cytostoma), and both have a reproductive process not directly to be subordinated to simple cell-division.

In the Acineta, special organs for the absorption of nutrition are present, pointing to a higher grade of development.

Amongst "Protista," the Noctiluca, Flagellata and Catallacta are separated by the development of flagella and cilia as a more highly differentiated group.

The Mycetozoa and Radiolaria, in the mode of the inception of nutriment and of locomotion, would approximate to this group; but the former, by reason of the peculiar complication of the process of development, assume an exceptional position; whilst the Radiolaria, on the other hand, by the possession of a "central capsule," as well as of true "cells" embedded in the body-sarcode, are raised to a degree of development and differentiation, elevating them considerably above the Polythalamia and Heliozoa, with which Häckel has combined them in the class of the Rhizopoda.

Thus for the present group—of the Protista, only the Polythalamia and Heliozoa—of the Protozoa, only the Amœbina and Monera would be left. These about correspond to the extent of the group Rhizopoda, as founded by Claparède and Max Schultze, and as generally received. The name "Rhizopoda" might therefore be retained for the group as a whole, but the authors suggest a new name therefor owing to the following reasons.

The name "Rhizopoda" takes its origin from the supposed resemblance of the pseudopodia to the roots of a tree. Such a comparison manifestly is inapplicable to the broad-lobed processes of a Difflugia or Arcella, and altogether loses its force applied to the flow and current of the body of an Amœba, or the uniform rotating motion of a Hyalodiscus. They

hence prefer to limit the use of the term to those organisms possessing pseudopodia of a branched "rootlike" character and to apply a name to the whole group more in accordance with them in their totality. Inasmuch as the common bond of union is the undifferentiated protoplasm, they suggest the comprehensive name Sarcodina (Sarcode organisms). As there does not exist any proof of a monophyletic origin of all living beings, but rather a polyphyletic descent must be regarded as more probable, this community of nature cannot be regarded as inherited from a common primitive form, but it is rather to be supposed that under certain similar chemical and mechanical conditions similar forms must have come into existence. Therefore, by the union of a considerable number of these lowest forms as Sarcodina, the authors do not mean to express a community of origin, but rather only that they all have attained to, and remain stationary at, an equally high or, better said, equally low, stage of development.

Be their origin what they may, one thing continues to force itself on my own mind, and that is the strongly marked *specific* differences which present themselves in the freshwater rhizopodous forms, both of inherent characters, habit, behaviour, and idiosyncrasies, but having referred to these in former papers I need not recapitulate.[1] I allude, of course, to "fully-grown" examples, in which their characteristic specialities are completely assumed; no doubt one sees in freshwater collections certain minute heliozoan and other types of a "nondescript" character, which one is compelled to assume, *ad interim*, may be "young" or undeveloped examples, possibly proceeding from subdivision or from zoospores of described species. It is possible, no doubt, that inasmuch as the freshwater forms are vastly more few than the marine, and the lacunæ or intervals between the forms wider, and hence standing *inter se*, more remote, they allow of their mutual differential characters being more readily perceived. In Difflugiæ, &c., the difficulty (as in other groups) is to know if certain forms be amongst those already described, not in seeing the mutual distinctions that present themselves, and in recognising this or that old friend or stranger (as it may be) when the examples are met with.

Hertwig and Lesser regret their inability to have carried their observations on to the marine forms, and to have submitted the Polythalamia to renewed researches, as bearing on their relation to the Monothalamia.

As the efforts of the authors were directed to the formation

[1] 'Quart. Journal Mic. Science,' n. s., vol. xi, p. 107; vol. xv, p. 129.

of groups, in which a considerable number of characters in common might have led to the supposition of a common origin, they still have refrained from trying to subordinate *all* the forms they met with to a single system. Efforts in this direction have as yet produced but artificial schemes. Thus, Claparède would separade Trinema and Euglypha from Microgromia and Lecythium, and would place Arcella and Difflugia far away, and in close proximity with Actinophrys and Heliozoa. Again, were there attributed to the contractile vacuole a high systematic value, Lecythia would stand more removed from Microgromia, Pleurophrys from Trinema, than the latter from Actinophrys and other Heliozoa. So, things manifestly related would be dissociated, forms standing remote would be combined.

The authors in their systematic distribution go on the following plan: they do not start from the consideration of the group as a whole, and from certain prominent characters, as do previous observers, but from the consideration of the individual organisms; and from an exact study of each form they proceed to combine individual species into genera, and these again into families and orders. But this plan they only so far follow where a considerable sum of common characters seemed to admit of their combination as one true to nature. Forms which would not allow of being so subordinated they prefer to allow to remain, *ad interim*, isolated, in order that such exceptions should not disturb the certainty gained with regard to certain groups of having only related forms in view. By this inductive method the authors arrive at the assumption that two "blood-related" groups become established, the Monothalamia and the Heliozoa. Nearer relations between them than would be expressed by the common subordination of both to the Sarcodina the authors do not hold to exist.

After the establishment of the foregoing two groups founded on a blood-relationship, they have combined those Sarcodina which remain over into a further group, in which, however, no blood-relationship seems to be expressed amongst the individuals thus brought together.

I may be allowed here to remark that, referring to the recent contributions of other observers besides Hertwig and Lesser, there appears to me to be a general fault in the formation of *too many genera*. I doubt not for a moment the individuality and specific distinctness of the forms brought forward, but it seems to me that each observer too often makes a "new genus and species" for each form whereas, for the present at least, it would seem more advisable, if the forms admit of it, to subordinate them to older

genera; nay, cases are not wanting where different observers would seem each to have named, as a new genus and species, one and the same thing. Such cases I would try to point out in the course of the *résumé*.

On the whole it appears preferable to commence our review of newly described forms with the Heliozoa, to pass thence to the Monothalamia and conclude with Hertwig and Lesser's new forms not belonging to either and grouped together simply as a "mixed lot." Necessarily it would be impossible to give the whole of the matter in full, as it would suffice to fill several numbers of this journal, and we must be content with an effort at an epitome and by reproduction of certain typical figures to afford home microscopists an opportunity to identify, at least approximately, such of these forms as they may encounter.

After giving a short historical *résumé* of the views of previous writers[1] on those "Rhizopods" referred to by them as "Freshwater Radiolaria," and who, on the whole, were inclined so to designate them, Hertwig and Lesser, approaching the consideration of the question with what right these debateable organisms can be regarded as appertaining truly to the "Radiolaria," propound first the query, "What are the conditions of organization characteristic of the Radiolaria?" for, as they rightly hold, upon the answer thereto would depend the reply to the further question, "How far these characteristic conditions of organization can be demonstrated in the 'Freshwater Radiolaria.'"

In accordance with Häckel's views as to the true (marine) Radiolaria, the following are the characteristics of that class:

1. They are multicellular organisms, nucleated, and containing imbedded in their protoplasm numerous perfectly independent walled cells.

2. They all possess a "central capsule," that is, a multicellular structure, posed in the centre of the body, mostly of globular figure and surrounded by a thick chitinous membrane.

3. Most of them possess yellow-coloured cells (for the most part imbedded in the outer sarcode), the so-called "yellow cells."

[1] Focke, 'Zeitscrift für wiss. Zool.,' Bd. xviii, p. 345; Cienkowski, 'Archiv f. Mikr. Anat.,' bd. iii, p. 311; Carter, 'Ann. and Mag. Nat. Hist.,' 3. Ser., vols. xii and xiii; Grenacher, 'Zeitschrift f. wiss. Zool.,' Bd. xix, p. 289; Greeff, 'Archiv f. Mikr. Anat.,' p. 464; Archer, 'Quart. Journ. Micr. Sci.,' vol. ix, pp. 250, 386; x, pp. 17, 101; xi, p. 107.

Hertwig and Lesser regard the first two as absolutely essential, rightly dismissing the so-called Coscinosphæra, Stuart[1] (referred by him to Radiolaria, but described as having no central capsule), as without doubt a Foraminifer, the authors come to the second question as above. This they at once answer for themselves in the negative, and prefer to denominate the so-called freshwater "Radiolaria" after Häckel as "Heliozoa." They then proceed to discuss the questions:

1. Are the Heliozoa unicellular or multicellular organisms?

This is a question as regards the freshwater forms not hitherto definitely taken up by any previous observers. It is true that Greeff, speaking of Acanthocystis, alludes to "vesicles containing a nucleus-like structure, and then offering the most complete resemblance to cells,"[2] and further on alludes to "manifest cell-like structures," but without expressing any view as to the relation of these to the build-up of this organism, or whether the observation has any wider applicability, except as regards *Actinosphærium Eichhornii*)where truly many nuclei coexist).

Hertwig and Lesser, however, are of opinion that with a few exceptions the Heliozoan individuals possess but the value of a single cell. They regard, in fact, the median globular (or ellipsoidal) body to be found therein as a *nucleus* with its *nucleolus*—the whole surrounded by a membrane ("Kernmembran")—its appearance and structure perfectly agreeing with the undoubted nucleus in Monothalamia and other Rhizopoda.

In this I believe they are perfectly right, and whilst in my own published remarks I have hitherto rather leant to the view of this central body being probably homologous with the "central capsule" of the Radiolaria proper than to be considered as a nucleus, it was still with hesitation—with a "so-called" before either designation.

An exception to the possession of but a single nucleus in the Heliozoa is formed by *Actinosphærium Eichhornii*. In it, as is well known, are imbedded numerous nuclei, without, however, any definite protoplasm territory being limited off around each. Whether there be expressed hereby a true "multicellularity" or not the authors naturally leave in doubt, and would rightly restrict that term to such forms as, with a multiplicity of nuclei, show an individualisation of independent portions of the protoplasm no longer in con-

[1] Stuart, 'Zeits. f. wiss. Zool.,' Bd. xxvi, p. 328.
[2] Greeff, 'Archiv f. Mikr. Anat.,' Bd. v, p. 480.

tinuity. In none of the Heliozoa is the high histological development known in Radiolaria attained.

2. Do the Heliozoa possess a "central capsule" or a structure homologous thereto? Are the (median) rounded bodies seen in their sarcode which have sometimes been regarded as central capsule, sometimes as "inner vesicle" (Binnenblase, Häck.), actually, indeed, organs so strongly demonstrative of their Radiolarian nature?

Hertwig and Lesser answer these questions with a decided negative.

For my part, as mentioned, I feel they are quite right. Nor has the nucleus alone been interpreted by various authors as "central capsule" or "inner vesicle" for likewise different regions of the body-mass itself have been so denominated.

In fact, to demonstrate the "unicellularity" of the Heliozoa and the nuclear nature of the central body is at the same time to show that the latter is not to be interpreted as central capsule.

3. Do the Heliozoa possess structures homologous to the "yellow cells" of the Radiolaria?

The yellow and green globules seen in many Heliozoa, *but not confined to them*, have been interpreted as equivalents of "yellow cells." But Hertwig and Lesser rightly point out they have no trace of cellular structure. In many cases they differ only in size, not in colour or apparent nature, from the minute granules distributed in the sarcode.

Perhaps till more is known concerning them they had better be simply regarded as pigment-granules; some of these attain sometimes a high colour, as in *Pompholyxophrys punicea*. Hertwig and Lesser regard such as stored-up nutriment; but whether their colour in any way depends on the food, or whether colourless at first, they afterwards during the digestive process acquire a colour, they would leave in abeyance.

Originate as they may, Hertwig and Lesser in any case rightly aver they are not *cells*, only constituents of the contents of cells.

To myself, their colour, average size, and usual quantity seem to a certain extent specifically characteristic; of two kinds of them, the green and the yellow, I would take the former to be "chlorophyll" (e. g. *Acanthocystis turfacea*, *Raphidiophrys viridis*), the latter to be oil-globules (e. g. *Acanthocystis spinifera*); very similar oily globules occur in various animal and vegetable organisms and in diatoms.

Thus argue these authors that *Heliozoa are no true Radi-*

olaria, as (most of all) Greeff has striven to show. They then pass to the consideration what value the remaining characteristics shared by the Heliozoa and Radiolaria in common possess, and how far these may point out the two classes as two groups, as it were, growing upwards from a common root; and some striking common characteristics, no doubt, do present themselves, the most prominent of which are the general figure of the body and the form of the skeleton.

The sphere must be regarded as the fundamental form of the Heliozoa (homaxial fundamental form—"homaxone Grundform," Häckel), for the formation of a stipes in some, pointing as it does, to a certain extent, to a differentiation of a posterior fixed, and an anterior free, extremity, may be regarded as but a secondary alteration, exerting no influence on the figure of the skeleton or the body itself. Now the same typically *homaxial* fundamental form holds good for the Radiolaria. Even the divergent forms presented by the Cystidæ, Discidæ, &c., may be referred to a differentiation of the body-axis, only some degrees further carried from the original typical globular fundamental form.

The question follows how far this agreement of body-figure points to affinities between the two groups, touching which the authors, whilst remarking that inner organization and its mode of development is generally admitted as of more importance than external configuration, record their own opinion that they do not hold the view of any close affinity between them, notwithstanding agreement in external form, and taking into consideration their differences of inner organization.

Just as little do they see reasons to subordinate the Heliozoa to the Radiolaria as regards the formation of the skeleton. No doubt there are in this regard parallelisms—thus Raphidophrys with Sphærozoum, Acanthocystis with Acanthometridæ, Clathrulina with Ethmosphæridæ,—*possibly* (?) Heterophrys with Spongodiscus. Still amongst Heliozoa there are skeleton-forms not comparable with those of Radiolaria—thus the globules of Pompholyxophrys (Hyalolampe)—the plates of Pinacocystis. In Radiolaria the portions of the skeleton for the most part lie partly or wholly immersed in the body-sarcode, and even sometimes penetrate the central capsule; in the Heliozoa, urge the authors, they form a globular envelope (Kugelmantel), at some distance from the superficies of the body, and whose individual pieces, as would to them appear, are held together only by protoplasm threads "emanating from the pseudopodia."

For myself I cannot still help thinking that there exists

an outer cloudy protoplasmic envelope forming a matrix whence radiate the spines, or in which lie imbedded the spicules; of this, however, more when the forms themselves come to be referred to.

The authors, however, point out as more important, that the presence of spicules or spines is a widely enough distributed characteristic in other groups; thus in Sponges, some Foraminifera—or of campanulate fenestrated siliceous tests, like the shells of Cyrtidæ (as pointed out by Häckel) in some pelagic Infusoria,[1] showing that resemblances of the skeleton may depend as much on analogy as on homology, and therefore give no ground for uniting two classes systematically, when the step cannot be supported by characters based on the structure of the soft parts.

The authors, therefore, arrive at the conclusion that, in the present state of our knowledge, there does not exist a single characteristic in the structure of the Heliozoa which would compel us to regard them as otherwise than remote relations of the Radiolaria. As yet the view that both Classes have originated independently, and each for itself, has the greater amount of evidence in its favour. But they admit that a study of organization alone would not decide the point, as only a comparative research into the development-history of the two Classes would lead to a certain decision. Unfortunately our knowledge as regards the latter, in both instances, is very defective; in the Heliozoa only encysting—fission—zoospores—are known, whilst in Radiolaria Cienkowski has described flagellate zoospores arising by subdivision of the contents of the central capsule. Still these observations, so far as they go, carry not much weight as regards the question, for quite similar zoospores occur in the Monothalamia,[2] and, indeed, in Mycetozoa and in Cienkowski's "*Monadinæ Zoosporeæ.*"

But nothing is known of the mode whereby a zoospore, in coming to rest, developes into a Radiolarian, and how out of a probably unicellular structure a protoplasmic mass, including many cell individuals, originates, and this knowledge alone can inform us whether the Heliozoa bear a relation to the Radiolaria comparable to that of lowly organized forms to more highly developed.

Hence the authors came to the above result; that is to say, it is possible that an affinity between Radiolaria and

[1] Häckel, "Ueber einige neue pelagische Infusorien," in 'Jenaische Zeitschrift,' Bd. vii, 4.

[2] Hertwig, "Ueber *Mikrogromia socialis*," l. c., p. 22.

Heliozoa may be demonstrable—demonstrated, at present, it is not.

The Heliozoa, then, are an independent Class, and the following would appear to be their characteristics.

They are unicellular, rarely (by multiplication of nuclei) multicellular (or multinucleated?) organisms. Their fundamental form is that of a sphere, whilst a very few are fixed by a stipes. The protoplasm, of which alone the soft part of the body is wholly composed, is in the greater number of forms differentiated into an endosarc and ectosarc.

The limitation of these layers is more or less pronounced; in *Actinophrys sol* they pass imperceptibly one into the other; in Actinosphærium the modification is complete; the limits seem most sharply marked off in Acanthocystis, Heterophrys, &c., but even in them it is a differentiation of the protoplasm, not a special membrane, which determines the distinctness of their contour.

In the endosarc constantly lie the nuclei; if the nucleus, as mostly, is single, it is more or less excentric; if there be numerous nuclei, they are irregularly scattered.

The ectosarc is characterised by the possession of contractile vacuoles (though not as yet demonstrable in all species). They mostly occur at given places, and are variable in number. In Actinophrys and others occur likewise simple vacuoles, which are more highly developed in the ectosarc, but may extend even into the endosarc.

The pseudopodia, serving, as they do, both to the capture of nutriment and to locomotion, are constantly thin and filiform, and originate all round the superficies of the globular body; they frequently reach a length several times exceeding the diameter of the body, are sometimes homogeneous, and sometimes granules pass along them slowly up and down. Ramifications or anastomoses sometimes, but do not usually occur, to some extent possibly due to their radial arrangement forbidding the opportunity for their mutual contact.

A speciality of the pseudopodia of many Heliozoa is the differentiation of their substance into an axis and cortical investment, to which, in Actinosphærium, Max Schultze was the first to draw attention; this extremely delicate axis reaches the length of the pseudopodium and passes down at least as far as the endosarc; it consists of a homogeneous substance dissolved by acetic acid. The authors regard this axis as a strengthening apparatus for the pseudopodia, and probably only due to a condensation of the protoplasm. They regard Greeff's comparison of it to the spines penetrating in

the central capsule of Acanthometridæ as but conjectural. In this I must wholly coincide with Hertwig and Lesser.

How far this central axis of the pseudopodia is extended amongst Heliozoa is questionable; if generally present it is certainly extremely difficult of detection.

The skeleton sometimes consists of a single solid and globular piece—sometimes (and more frequently) of numerous loosely combined pieces, globularly distributed. The latter are mostly bacillar, and if tangental in position are denominated "spicules," if radial "spines." Some (Actinophrys, Actinosphærium) are destitute of any skeleton.

The locomotion of a Heliozoan is a very slow one; it balances itself on the apices of the pseudopodia, and by help of their contractions rolls (very slowly) onwards.

Any propagation known is asexual—simple fission of the body-substance, distinguishable, however, into two modes: that is, division of the naked body or division within a cyst. The individual portions originating in the latter manner become once more themselves encysted.

The daughter individuals arising by division either pass at once into their persistent form or go through a zoospore stage, assuming an ovoid figure, and developing at one end two flagella. In Ciliophrys, Cienk., the whole organism passes directly into a zoospore condition. None of these modes of development seem to have a generic or specific significance. Thus from the cysts of *Actinophrys sol* come directly young individuals of Actinophrys; on the other hand, zoospores from the cysts of Clathrulina. The subdivision of the non-encysted Clathrulina body sometimes produces zoospores, sometimes directly young Clathrulinæ, establishing themselves at once on leaving the mother individual.

Heliozoa Askeleta.

The great character of these must be regarded the spherical figure, combined with the absence of any skeleton. So great stress do Hertwig and Lesser lay on the former character that if a rhizopod, though *sometimes* globular, habitually assumes an irregular figure, and during locomotion undergoes amœboid modification of contour, they would exclude such from this subgroup. Thus they would not refer Nuclearia, nor Leptophrys, nor Vampyrella, even if it had a nucleus, hither; and in this I feel they are perfectly right. There is a steadiness, so to say, of form, or there is a characteristic polymorphism evinced by each of these lowly organisms which seems wonderfully inherent.

Here belong only the genera Actinophrys and Actinosphærium.

Actinophrys sol, Ehrenb.

At any great length here to describe this, one of the longest known and most widely distributed of Heliozoa (nay, Hertwig and Lesser take the marine form *A. oculata*, Stein, for identical with that of the freshwater), would be out of place, at least unnecessary. Yet *A. sol* had been found and recorded over and over again by observers everywhere before the possession of a nucleus was suspected or noticed. Even Häckel supposed it to be a moneron. The first to detect it was Grenacher;[1] previously, indeed, the structure was described in *A. oculata*, Stein,[2] and confirmed by Carter.[3] It is perhaps curious that it should so long have escaped observation, as in no freshwater Heliozoan is the nucleus more easily perceptible. Of course, indeed, it is easy enough to refind anything *when one knows it is there.* It is some years since I first noticed it, and had a sketch made of it as regards a very large and fine but rather uncommon form of *A. sol*, when I accidentally found that Grenacher had been in advance of my discovery; I then saw that it was present in the ordinary, more minute, and more common form. Grenacher was inclined to regard it, however (as at the time I was rather of opinion myself), as "central capsule." This structure possesses a globular figure, with a sharply-marked contour and a smooth surface; the contents appear to be of palish-blue colour and finely dotted, though seemingly of homogeneous consistence. Grenacher saw in this structure seemingly but a hollow sphere containing a homogeneous plasma, coagulating under acetic acid; but as Hertwig and Lesser regard it, this inner "coagulated" portion must be taken as *nucleolus.* In the normal state the latter is not readily seen.

In some researches which I instituted on this nuclear body I find that iodine and sulphuric acid produced little effect as regards consistence of the nucleolus; the outer wall becomes more black and decided, the "contents" reddish. Iodized solution of chloride of zinc rendered the nucleolus plain, but best of all Beale's carmine fluid rendered it apparent; the nucleus became a lively red and the nucleolus

[1] Grenacher, "Ueber *Actinophrys sol*," in 'Verhandl. der Physic. med. Gesellsch. zu Würzburg,' Bd. 1 (1868), p. 170, t. III.

[2] Stein, 'Die Infusionsthiere auf ihre Entwickelungsgeschichte untersucht.,' p. 160.

[3] Carter, 'Ann. and Mag Nat. Hist.,' vol. v, p. 277.

a deep red. According to my measurements the diameter of the nucleus was about $\frac{1}{1250}''$, of the nucleolus about one half that measurement; however, as the examples were very large, it is possible these may be, as a rule, over the average of the dimensions of this structure.

I must now entirely agree with Hertwig and Lesser that this central body is of nuclear nature, not the homologue of "central capsule;" and though I once in drawing attention to the corresponding structure in *Acanthocystis spinifera*, suggested that the inner body might be comparable to the "Binnenblase" rather than to nucleolus, I think that the latter explanation is doubtless the true one, and must hold with Hertwig and Lesser that the Heliozoa of which Actinophrys is the first, the simplest type, thereby lose all claim to rank as a freshwater group of Radiolaria.

After giving some of the views of previous observers as to the action and possible function of the large peripheral pulsating vacuoles, which the authors believe do not decide their being of excretory nature, they proceed to the consideration of the pseudopodia and agree with Grenacher in ascribing axile threads thereto, similar to those of Actinosphærium. These can be more readily discerned under the action of dilute acids or alkalies than in the natural state, and indeed, according to Grenacher, their continuations into the body, may be seen as far as the "nucleus" ("centrales Bläschen" of Grenacher), more plainly than permeating the pseudopodia themselves, but Hertwig and Lesser have failed in detecting them passing into the body. They argue that these linear threads have indeed nowhere been seen to reach the nucleus or have any relation thereto, but on this see observations on Acanthocystis.

Like many other Heliozoa, as is well known, two or more examples of *A. sol* can become fused together and can subsequently become dissociated, and this is very frequently to be seen. The authors believe the phenomenon does not represent a previous growing larger of examples followed by self-division, but is truly a fusion of before separate individuals. They do not think it has anything to do with any reproductive process, but is quite accidental, possibly enabling the group to capture and digest larger prey, more manageable for their combined powers, than for the unassisted efforts of a single Actinophrys.[1]

It is some time since that I first met with an extremely large Actinophrys, habitually densely charged with large and bright green chlorophyll-granules, quite like those of *Acan-*

[1] 'Cienk. Archiv,' Bd. i, 227.

thocystis turfacea or of *Raphidiophrys viridis*. Latterly I have not been able to meet with it. I have indeed sometimes supposed in the field that I had encountered it, but when I examined the gathering at home what I had really taken has turned out to my disappointment to be *Acanthocystis turfacea*. Of all freshwater Rhizopoda I think this *green* Actinophrys appears to me to form the most beautiful object, due as much, if I might so say, to its *energetic* behaviour as to its fine size, very large pseudopodia, and bright colour: I allude to the very vigorous play of its numerous vacuoles, perpetually in action so much so as to impart an everchanging *frothy* appearance to the whole disc, almost comparable to the carrying on of a kind of ebullition. The pseudopodia in this form are extremely long, rather stout, sometimes some of them showing an expansion of a fusiform figure along their length, formed of granular sarcode; the play of granules up and down very vigorous. Sometimes, though rarely, one could see an inosculation of two or even three of the pseudopodia; three starting from the periphery separately might be found joined about halfway along their length and thence continued as a single one to the apex. The periphery of the large contractile vacuoles sometimes showed a pilose appearance, being covered all over by a number of closely set delicate hair-like pseudopodial (?) processes. This form is a rather hungry one, incepting food sometimes of large dimensions; thus one could see it sometimes engulph one of the joints of a neighbouring Mesocarpus (which form rather long objects), gradually embracing it until a firm hold of the joint was attained, when it would naturally break off at the suture between it and the adjoining joint, whereupon the captured one would next be seen to be wholly surrounded by the vacuolar sarcode of the Actinophrys. This form occurred in the county Westmeath and would well deserve a close study, but it seems to be very scarce. I am not at present inclined to consider it as specifically distinct from *A. sol*, for as is well known the presence of chlorophyll-granules does not appear to be a sufficiently constant character to be accounted as of specific value — thus *Amphizonella vestita* (Archer, olim), better *Cochliopodium vestitum* (Archer) (= *Cochliopodium pellucidum* and *C. pilosum*, Hertwig and Lesser, which to me appear to be but forms of one and the same species, of which, however, more hereafter), as well as *Acanthocystis turfacea* (Carter), and even *Raphidiophrys viridis* (Archer), all can occur colourless, and I at present am not aware of any further important character, beyond its large size, to separate the fine Actinophrys in question from the

ordinary *A. sol.* It was in the form alluded to that I noticed the evolution of zoospores as recorded in the minutes of the Dublin Microscopical Club.[1] These were in length $\frac{1}{3000}''$, in breadth $\frac{1}{5000}''$, pyriform, bearing two flagella of different lengths at the pointed end, the longer about as long or one and a half times as long as the body, which showed no "eye-speck" and sometimes contained one of the chlorophyll-granules. They seemed to be given off directly from the body-substance; as to their fate I regret I know nothing.

Ciliophrys infusionum, Cienkowski (fig. 1),

Is the name given by the author[1] to a Heliozoan found in long-standing infusions and amongst colourless Oscillatoriæ, Leptophrix-forms, &c., seeming to present no very strong "structural" generic character to separate it from Actinophrys. It has the habit of *A. sol*, but it is considerably smaller. In the regular distribution of the granuliferous pseudopodia, in the possession of a central nucleus with nucleolus, in the often occurring vacuolar (frothy) consistence of the protoplasm, especially at the periphery, and in the mode of inception of foreign bodies, both forms coincide. The only distinction, apart from size, relates to the contractile vacuoles. In *A. sol* these are large during the diastole, very considerably and prominently projecting—contracting in a wrinkled manner during the systole. The present form possesses one to three very small contractile vacuoles (which judging from the figure, do not seem to "wrinkle").

Now, I myself would be much disposed, were it not for the circumstance noted below, here to adduce the present as a case in point of the establishment of at least *one genus too many*. The organism seems so far as "body-structure," taken as a whole, is concerned, to be to all intents and purposes, an Actinophrys. The author himself, indeed, admits that the establishment of a new genus and species therefore is, at best, but arbitrary.

The author regards the present form as the only one of naked Heliozoa known to produce zoospores. It may, however, call to mind my own cursory observations[3] on a large chlorophyll-bearing form of *A. sol* (such it must be considered at least for the present) in which Zoospores were given off one by one from the living (unencysted) organism.

[1] 'Quart. Journ. Micr. Sci.,' vol. x, New Ser., p. 306.
[2] Schultze's 'Archiv. für Mikrosk. Anat.,' Bd. xii, p. 29, t. v, figs. 26—43.
[3] 'Quart. Journ. Microsc. Science,' vol. x, p. 306.

In Cienkowski's form, however, the whole body-mass became directly converted into zoospores, which is very remarkable. May *this* circumstance, then, be sufficient to distinguish the two forms not only as different species but as separate genera?

In the course of from twenty minutes to an hour under the author's eyes the change became completed. During the gradual transformation of the body a modification of the contents was noticeable—originally coarsely granular they became gradually of a mucoso-homogeneous consistence, the nucleus more and more distinct at the same time the pseudopodia disappeared, the body gradually assumed an ovoid form and the nucleus removed from its central position towards the end, at which were soon noticeable one or two cilia (flagella), by action of which the whole body assumed at first a tremulous motion, gradually becoming more vigorous, until at last it passed into a rotation on its longitudinal axis and an advancing movement. The author could not follow out its further fate, these zoospores ever vanished into the "dirt" and algæ around (as such perverse things *will!*). The zoospore had an ovoid figure, often appearing drawn out into a stout process; in some examples the author saw a little posterior contractile space, besides usually a few non-contractile vacuoles.

The near affinity to *A. sol* was further evidenced by the fission into two of a single individual, and again by the fusion into one of several individuals. If a cluster of such "conjugating" individuals passed into the zoospore state, each lobe of the group, that is each component, previously distinct, individual became a zoospore. Just the same if undergoing division, each segment became a zoospore. But further, the zoospores themselves could become "conjugated" or fused together, each portion of the compound body possessing its nucleus and cilia, and the whole assuming a roundly triangular figure. Whilst the author could trace results no further, he sees no ground in the fact of fusion as described to base any assumption that it is to be at all regarded as a sexual act.

Actinosphærium Eichhornii (Ehrenb.)= *Actinophrys sol*, Kölliker.

The structure of this common Heliozoan is now too well known to need any copious description here. Greeff regarded the line of demarcation between the endo- and ectosarc, apart from their different transparency, as produced by "a thin

homogeneous protoplasm-zone, surrounding the whole inner space like a thick membrane." To this Hertwig and Lesser are opposed, nor does there really appear any such distinct separating layer, however thin, and the seeming boundary is due only to a somewhat sudden alteration of the size of the vacuoles and the varied thickness and degree of granulation of their walls. The vacuoles of the outer region in small individuals form but a single stratum, in larger several occur superimposed, and more or less radiately arranged. In the endosarc, the vacuoles are quite irregular. In very young individuals there is no differentiation of endosarc and ectosarc perceptible, and this becomes by degrees more and more pronounced in larger examples. But Greeff went further and explained the endosarc as "central capsule," manifestly an erroneous conclusion. The central capsule of the Radiolaria proper is composed of firm doubly contoured chitinous membrane, but here there is no coat of any sort around the middle region.

Moreover the central capsule is an organ of reproduction, in so far that by subdivision of its contents zoospores are produced serving to reproduction. It does not play a part in assimilation, which is exclusively performed by the extra-capsular sarcode. On the other hand the endosarc of Actinosphærium is the peculiar seat of the digestive function, foreign bodies rarely being found in the ectosarc region, except *in transitu* inwards.

As is well known, sudden external forces cause retraction of the pseudopodia and partial obliteration of the external vacuoles; after rest the organism slowly reassumes its ordinary appearance.

The marginal contractile vacuoles differ in nothing (are scarcely even as a rule larger) than those of of *Actinophrys sol.*

The nuclei are very numerous, possibly considerably over 100 in large examples. Carter supposes he must have observed as many as 400 in one individual: they occur only in the endosarc, and, indeed, mainly confined to its outer part. They form globular or subelliptic bodies, with rounded nucleolus, and, indeed, quite resemble those of *A. sol*, and, for that matter, those of many other Rhizopoda. Max Schultze records an observation of three to four, up to as many as twenty corpuscles (*query* nucleoli?), as contained in the nuclei. What their significance may have been appears doubtful to Hertwig and Lesser. Carter,[1] however,

[1] Carter, 'Ann. Nat. Hist.,' p. 282.

denominates these "spherical bodies" within "reproductive cells"—the former, no doubt, Schultze's nucleoli, the latter the true nuclei.

Corresponding to the large size of this species the pseudopodia are very large and long; they are developed all over the globular body; as to the axile threads, as before mentioned, Hertwig and Lesser regard them as mere strengthening apparatus for the pseudopodia, and are opposed to Greeff's view as regards their nature. Greeff would regard them as skeleton-spines; but true spines are rigid, withstand the action of acids; on the other hand, these slender linear axes are flexible, and disappear under reagents. It is not even clear that they may not be withdrawn with the pseudopodium and reproduced *pari passu* therewith. (See remarks further on.) Again, in regard to the true spines of other forms, the pseudopodia do not form a complete investment around them, but they lie apart from the body in an external stratum.

It is, of course, needless to urge that Actinosphærium is a true Heliozoan, not a Radiolarian. Its affinity, and, indeed, resemblance to *Actinophrys sol* is considerable, and they have often been confounded, as by Kölliker, and, indeed, Wallich has sought to show they are not distinct; but this is no more than he has done in other instances equally untenably. In the fully-grown Actinosphærium, besides the great difference of size, the nuclei are numerous, the two regions, both alveolar, distinctly marked; in Actinophrys, the nucleus is single and the endosarc homogeneous, passing by degrees into the vacuolar ectosarc.

It is true that young forms of Actinosphærium are no larger than some examples of *Actinophrys sol*, but there is, to my own eyes, always something about the youngest and least developed specimens of the former that tells at once which species it belongs to. As Hertwig and Lesser point out, the alveolæ of the former are always larger than in the latter; the pseudopodia of the young specimens of the former are often very few and short, but are always numerous in the latter. I must, for my part, wholly concur with Hertwig and Lesser that the two forms are essentially specifically distinct, and further that they ought to be maintained as types of two distinct genera, in accordance with Stein and Haeckel.

Professor F. Eilhard Schulze records some valuable and interesting observations on the structure, and subsequently confirms previous observations on the development of *Actinosphærium Eichhornii*, Stein = *Actinophrys Eichhornii*,

Ehrenb.[1] This form, under the erroneous name of *Actinophrys sol*, was long ago the subject of study by Professor Kölliker,[2] whose account of this now widely known form, subsequently enlarged by Stein[3] (who correctly placed it in a genus distinct from Actinophrys), is briefly referred to by Schulze (l. c.), and hardly nowadays needs any further recapitulation here. The axile threads in the pseudopodia, discovered by Max Schultze,[4] are referred to above in connection with Hertwig and Lesser's observations.

Some remarks on this fine form were published by Greeff,[5] who believed he had detected, not two only, but in reality four, concentric strata in the body-substance—(1) an extremely thin, granuliferous stratum surrounding the whole body, possessing a slow current, and continued directly into the soft pseudopodial cortex; (2) the clear alveolar stratum following thereupon; (3) a comparatively thin *homogeneous* protoplasma-layer posed between ecto- and endo-sarc, mutually separating these and surrounding like a thick membrane the whole inner space; this he hence directly compares to and even designates it as the homologue of the central capsule of the Radiolaria; and (4) the minutely reticulate, dark, richly granuliferous endosarc. Professor Greef further regarded the pseudopodial axes as spines, and thought that they penetrate the "central capsule" of soft organic substance, and end inwardly, in the endosarc, with a blunt clavate extremity.

Professor Schulze sees no reason (nor does there, I fancy, really any such exist) to give up Kölliker's original view of but two strata—endo- and ecto-sarc—nor does he perceive Greeff's external cortical zone, nor the assumed intermediate layer. Schulze's own observations as to structure seem to coincide with those of Hertwig and Lesser. The sarcode surrounding the axes of the pseudopodia is but a continuation of that forming the ectosarc, and in some very broad ones even sometimes, near the base, may, like it, show alveolæ. Granules can sometimes be seen, carried by the slow current which prevails, to pass from the ectosarc region to the "cortex" of the pseudopodia and return therefrom back to the ectosarc. From this it follows that Greeff's presumed distinct external layer has no foundation in fact.

[1] Schultze's 'Archiv,' Bd. x, p. 328.
[2] 'Zeitschr. f. wiss. Zoologie,' Bd. i, p. 198.
[3] Stein: 'Die Infusionsthiere auf ihre Entwicklung untersucht.' (1854), p. 151.
[4] M. Schultze: 'Das Protoplasma der Rhizopoden und der Pflanzenzellen' (1863).
[5] Greeff, in 'Sitzungsberichte der Niederrheinischen Gesellschaft für Natur- und Heilkunde,' Bonn, Jan., 1871.

After a copious description of and dissertation on the various views as to the numerous nuclei of this form, Schulze passes to the pseudopodia with their "axes." The curious mode of ending of the latter passing in through the endosarc, discovered by Greeff, is confirmed by Schulze, as well as by Hertwig and Lesser. Max Schultze recognised them but as a peculiar form of contractile plasma, not as spines, in which Hertwig and Lesser, as mentioned above, coincide; but Greeff, followed by Eilhard Schulze, regards them as delicate, cylindrical "spines," comparable to "skeleton-pieces," whilst Greeff described them (as mentioned) as penetrating into the endosarc. Schulze avers that normally they only reach as far as that region, and, as it were, stand thereon.

This is certainly, so far as I myself can see, all they ordinarily appear to do, nor do they even seem to have the power to be retracted into it. When an example of this form is first transferred from a bottle and placed on a slide it often appears with the pseudopodia "tossed" and curved about every way, "dishevelled" looking, nay, occasionally inosculating, but soon they begin to stand out stiff and "trim." Were these axile threads rigid "spines," comparable to those of, say, an Acanthocystis, this could hardly happen.

Schulze, indeed, attributes to them a great elasticity, a capability of being bent and even, under some circumstances, being fused. They appear to consist of an organic albuminoid substance. M. Schultze had observed two axes in one pseudopodium, indicating, of course, the fusion of two adjoining and previously independent pseudopodia.

After describing the pulsating vacuoles the author proceeds to the description of the observations touching the reproduction of this form.

The author in the first place refers to the older observations of simple division into two and of the fusion into one of several individuals, both phenomena not unfrequent—nay, the latter can be artificially effected. Thus Cienkowski narrates the result of keeping on a slide in a drop of water an Actinosphærium composed of several fused individuals, as consisting in the withdrawal of the pseudopodia, the disappearance of the alveolar nature of the body, the whole becoming dark, finely-granular mucus, interspersed with numerous vacuoles, and surrounded by a clear, thickly-fluid envelope; in about seven hours the mass broke up into several dark balls, each surrounded by a colourless mucous envelope, acquiring subsequently a sharp contour surrounded by a membrane standing off at some distance; the remaining

substance, in which the balls were imbedded, gradually disappeared. Schneider afterwards[1] confirmed this observation, carrying the observation on to the development of young Actinosphæria; but this observer saw formed around so many pairs of balls, each of which contained eight to ten nuclei (of the same kind as the fully-grown Actinosphærium), a firm elliptic cyst, within which again each ball of each pair acquired an outwardly rough, inwardly smooth, thick-walled, special cyst, the common outer cyst bursting by and by. The thick wall of the inner cysts, according to Schneider, was composed of numerous siliceous pieces, having between them little lacunæ. From July to December these remained unchanged; they then showed the numerous nuclei had disappeared, and in the middle of each only one nucleus with nucleolus. Up to the beginning of May they remained in this condition; the walls of the cysts then burst, and there came forth a little Actinosphærium with a number of nuclei. The previous disappearance of the numerous (eight to ten) nuclei and the subsequent presence of only one was explained by Schneider as due to fusion thereof; and he attributed thereto the character of an act of fertilisation; hence he conceives that from the "ovum" (Ei) enclosed in the siliceous cyst, proceeds, by a process of cleavage of its before single nucleus, a young multi-nucleated animal; this grows and feeds and again copulates with other individuals, the special sexual act being the conjugation of the nuclei, whence proceeds the ovum-cell capable of development.

Greeff likewise suggests, as only very probable, a formation of embryos taking origin from the numerous nuclei of the "central capsule." He saw proceed from a great number of dead Actinosphæria a very large number of very minute Amœbæ, each with a nucleus and a contractile space, after the disappearance of which several little vacuoles appeared in lieu. These Amœbæ shortly passed into a resting state, assuming a globular or pyriform figure; these afterwards became changed into a flagellate zoospore.

Schulze's own observations agree generally with those of Cienkowski and Schneider, though differing from the latter in some points.

The pseudopodia became shortened and eventually wholly retracted, their axile threads becoming less distinct and gradually disappearing (proving surely it is not a "spine"), their cortical layer becoming drawn into the alveolar paren-

[1] Schneider: "Zur Kenntniss der Radiolarien," in 'Zeitschr. für Wiss. Zool.,' Bd. xxi, p. 507.

chyma of the ectosarc, the whole body becoming contracted into an irregularly rounded or ovate figure, the endosarc gradually losing its alveolar structure, and becoming condensed into a compact darker granular mass, and acquiring an almost smooth sharp limitation from the still alveolar clear ectosarc; now began the excretion of a rather thick, gelatinous envelope, showing here and there some concentrically stratified layers of granules, a few such here and there on the periphery; at other places appearing destitute of them, but not very sharply bounded.

Under favorable circumstances a number of clear spots were perceptible in the opaque endosarc, indications of so many nuclei.

In about half a day the first sub-division of the whole mass, along with the gelatinous envelope set in, and continued by binary sub-division, so that in from one to two days, say 10 to 30 subsequently approximately globular bodies resulted, though at first (or even continously) somewhat flattened by mutual contact, each enveloped in, as it were, its share of the simultaneously sub-divided alveolar cortex, *now*, however, having lost its alveolar structure. Imbedded in the centre of each there now appeared a single nucleus, which, when extruded by pressure, along with the granular body-substance, showed its large, dark nucleolus.

Thus Schulze's observation does not at this point concur with Schneider's, who found the sub-divided portions multinucleated, not single nucleated, that is to say simple cells, and who avers these latter to be enclosed in twos within a solid oval cyst. Still Schulze confirms Schneider so far that the clear cortex becomes ultimately silicified, but could not agree with him that this envelope was made up of siliceous pieces with intervening spaces, but it rather appeared as a membrane with either vacuities therein or external depressions. The inner mass sometimes showed slight temporary retrocessions from the wall, soon disappearing probably to reappear at a new place.

The author's train of direct observation became here broken, yet from renewed examination of other specimens he records further as regards the young animal, after emergence from the siliceous capsule, that it consists of a very thick, clear, alveolar ectosarc, not as yet very sharply differentiated from the sparingly granular endosarc, which latter encloses but a single clear nucleus of quite similar appearance to the multiple ones of the fully-grown examples; the pseudopodia are of greater tenuity than when fully-grown, the axis scarcely perceptible owing to its delicacy. During the following

summer months the young animals in the author's "little aquarium" continued to grow in size, and the nuclei to increase in numbers, most probably by division, and all the mature characters became by degrees assumed—thus closing the cycle of development.

Whilst thus Schulze's observations in the main coincide with those of Cienkowski and of Schneider, they would seem to fail to establish the views of the latter observer that a copulation of distinct animals is followed by a subsequent act of fertilization by means of conjugation of nuclei within the cyst.

Still the fusion of several individuals is no very uncommon phenomenon, and during the encysted state a diminution of the number of nuclei no doubt would seem to take place; Schulze, however, would suggest that is is more likely they may themselves perish than that the lessening of their numbers may be due to becoming mutually coalesced.

Professor Schulze refers casually to a form encountered by him in a bason of the Botanic Garden in Gratz, agreeing in most points with *Actinosphærium Eichhornii*, but is considerably smaller—the largest being $\frac{1}{10}$mm. in diameter—it likewise always showed but one pulsating vacuole, and lastly the limits between endo- and ecto-sarc was not so sharply marked off as in the ordinary typical form.

One most suggestively suppose that this was but a young and but partially developed representative of the ordinary form.

Touching the power of spontaneous fusion of the body-substance of several distinct examples of *Actinosphærium Eichhornii*, I might just momentarily call attention to the remarkably large sarcodic patches or masses met with by myself on two or three occasions in some deep pools in the county of Westmeath, and which *seemed* to have most likely so originated.[1] There were no pseudopodia, no differentiation of the two kinds of alveolar region, but the whole was uniformly alveolar with numerous nuclei, and an outer rather narrow, clear, hyaline, homogeneous, sharply bounded mobile border (ectosarc?), comparable to the corresponding region of an Amœba was present; the great patches, sometimes, as much as $\frac{1}{8}$ or even $\frac{1}{4}$ of an inch in diameter, when pressed out under the weight of a covering-glass, occasionally appeared broken into more or less large *holes* capable of recoalescing and thus becoming obliterated. Perhaps these indeed but represented the preliminary state of reproduction of this species described by the foregoing authors, and the sarcode masses

[1] 'Quart. Journ. Micros. Sci.,' n. s., vol. xi, p. 101.

would then be mainly remarkable only for their inordinate dimensions, and to form which would have required the co-operation of a very large number of individuals. But there was not a trace of pseudopodia, still less of "axes"; the whole had a certain degree of "flow," whilst the hyaline smooth margin here and there pushed out great convex (almost, when very much projected, hemispherical), wave-like expansions, like some Amœbæ, but with exceeding slowness. There seemed, however, to be evinced no tendency whatever to become encysted. This therefore still remains a problematic production.

In a future number I propose to continue the consideration of the newly-described Sarcodina, as recorded in the various memoirs.

The Process *of* Aggregation *in the* Tentacles *of Drosera rotundifolia*. By Francis Darwin, M.B. (with Plate XXIII.)

The term "aggregation" is applied by my father[1] to the changes which are produced in the tentacles of *Drosera*, and in certain parts of other plants, by the following agencies:—

I. The mechanical irritation of the glands by repeated touches or by prolonged contact with organic or inorganic substances.

II. Similar mechanical irritation transmitted from the glands on the disc to other glands on the same leaf.

III. The absorption of certain fluids such as solution of carbonate of ammonia, infusion of raw meat, &c.

IV. Heat.

V. Osmosis caused by immersion in glycerine, &c. I do not feel certain that this form of aggregation is identical with that due to the previously mentioned agencies, and it will not be further alluded to in the sequel.

The phenomena connected with these changes have been fully described by my father; the following remarks are merely intended to bring forward a few questions connected with this subject which have not at present been answered, and which are of importance to any one forming a judgment on the essential nature of process.

Aggregation occurs both in the pedicel of the tentacle and in the gland which surmounts it; the present paper

[1] 'Insectivorous Plants,' Ch. iii.

deals with the process as it occurs in the former of these parts, where it is far more easily observed.

The pedicel is composed of elongated cells of various lengths and of about ·016 mm. in breadth; the cells forming the upper and middle portions are filled with a crimson fluid (fig. 1), which is, however, absent from dwarf leaves grown in very shady places (figs. 9 and 10), and is absent from the lower cells of all the tentacles (fig. 8). The protoplasmic currents which may be observed in the cells vary much in complexity; either flowing round the cell-walls as described by my father;[1] or forming a complicated flowing network like that seen in the staminal hairs of *Tradescantia*, &c. This network is well seen in the tentacles of pale dwarfed leaves which grow almost hidden in moss; the current almost invariably flows from one chlorophyll body to another (when these are present), so that they are connected together by a most beautiful moving network. In other cells the currents are simpler as shown in figs. 1, 9, and 10. Neither my father nor myself have ever been able to make out a nucleus; and this forms a marked distinction between the flowing network of *Drosera* and that in the hairs of *Tradescantia*, *Cucurbita*, &c.[2] Well-formed chlorophyll bodies are found in the lower cells of the tentacle, in the middle cells they are dwarfed and yellow and do not contain starch, in the extreme upper cells close under the gland they are again found fairly developed. Highly refracting globules which appear to be oil-drops are occasionally seen floating in the coloured fluid and carried round by the currents.

The change from the normal state to one of aggregation is extremely striking; various forms and stages of aggregation are shown in figs. 1, 2, 3, 6 and 7. Instead of containing a homogeneous purple or crimson fluid the cells contain variously shaped masses of crimson matter suspended in almost colourless fluid. "The change is so conspicuous that it is visible through a weak lens or sometimes by the naked eye."[3] The crimson masses are now seen to undergo most remarkable changes both in form and position; the rapidity with which these changes occur varies with the nature of the exciting cause and the stage of aggregation in which the tentacle is; sometimes the changes are only to be detected by drawing a given mass and examining it after an interval of several minutes; in other cases they are so conspicuous as to

[1] 'Insectivorous Plants,' p. 38.

[2] According to Sachs ('Handbook,' p. 38) the nucleus disappears when the streaming commences in the sacs of the Characeæ.

[3] 'Insectivorous Plants,' p. 39.

be seen at once. This was the case with the cell shown in fig. 6; the appearance of the object changed as it was being drawn, so that it would have been impossible to draw the whole cell. By whatever cause aggregation is induced, it commences in the gland and spreads from cell to cell *down* the tentacle. On the other hand, when, on the cessation of the stimulus which has induced aggregation, the masses of crimson matter gradually dissolve and the cells become once again filled with homogeneous crimson fluid, the process of dissolution travels from cell to cell *up* the tentacle.

The above brief description may serve as an introduction to a discussion in which the various features of the phenomenon will be considered in greater detail.

The first questions which arise are—What is the nature of the aggregated masses, and with what parts of a typical vegetable cell are they homologous?

In the course of his description of the aggregated masses, my father remarks :[1] "Their movements are rather slow, and resemble those of Amœba or of the white corpuscles of the blood. We may, therefore, conclude that they consist of protoplasm." And in his subsequent discussion he always speaks of the aggregated masses as protoplasm. My father's assumption will appear justifiable to many; nevertheless more than one eminent physiologist have expressed doubts on this point. Professor Ferdinand Cohn in his review of 'Insectivorous Plants'[2] always speaks of aggregation as a "massing together (Zusammenballung) of the red sap" and never calls the masses protoplasm. Any opinion even hinted at by so eminent an authority deserves the most careful consideration. And since the question whether the aggregated masses are condensations of protoplasm or precipitations of cell-sap is of the utmost importance in determining the true nature of the phenomena of aggregation, it seems worthy of discussion. Before entering on the question it must be premised that the phenomena cannot possibly be considered as purely a mechanical or chemical one, like the running together of oil-drops into larger drops; the minute and continuous changes of *form*, and the separation into secondary masses absolutely negative such a view. Moreover the changes are subject to the general conditions to which protoplasmic movements are subject. It follows therefore that the changes not being mechanical must be vital, *i. e.* due to the movement of living protoplasm. If, then, we accept Professor Cohn's view that the masses of aggregated matter are passive co

[1] P. 40.

[2] 'Deutsche Rundschau,' ii, 9, 1876, p. 454.

densations of cell-sap incapable of originating movement, it follows as a necessary corollary that the movements are impressed on these passive masses by protoplasm of some kind external to them. We can therefore state the two opposing theories to be discussed, thus:—

I. My father's view, that the aggregated masses consist of protoplasm, and that their movements are simply due to their own contractility, excited by various external agencies.

II. Professor Cohn's view, which appears to be that the aggregated masses consist of condensations of cell-sap, and as a necessary corollary that the movements are impressed on the masses by some kind of protoplasmic action *external* to the masses.

The received notion of the structure of a typical adult vegetable cell is that within the cell-wall there is a sac of protoplasm enclosing the cell-sap, and sending prolongations in the form of "plates or threads," which traverse the cell-sap cavity in various directions; the nucleus may be described as differentiated protoplasm. The typical cell may, therefore, be said to contain two kinds of protoplasm.

As far as the present discussion is concerned Strasburger's classification of vegetable protoplasm[1] confirms this statement, although in reality he makes a third variety, his "couche membraneuse," which includes the peripheral or limiting portion of a protoplasmic mass.

A cell of Tradescantia obviously presents the two main varieties of protoplasm, namely, those which form the current and the nucleus, the rest of the cell being occupied by purple cell-sap. But if my father's view is correct, the Drosera cell not only differs in having lost its nucleus, but also in possessing another variety—a coloured protoplasm diffused throughout the cell, and distinct from the granular protoplasm which forms the current.

Professor Strasburger remarks "that the separation of the protoplasm into the granular plasma, the membranous layer, and the nucleus signifies a division of labour, in such a way that the nucleus governs the molecular phenomena connected with the genesis of cells, while the membranous layer is charged with the peripheral limitation of the whole, and the granular layer (or plasma) with nutrition."[2]

Now, the cells of the tentacle of Drosera, besides the vital functions common to all cells, have special ones to perform, namely, absorption of a special kind of food and the

[1] 'Sur la Formation, &c., des Cellules,' 1876, p. 263.
[2] Loc. cit., p. 263.

transmission of peculiar stimuli. It is not, therefore, surprising that a different form of protoplasm should be found in them. The origin of chlorophyll bodies in the vegetable cell is quite in accordance with Professor Strasburger's view; for, as Sachs remarks,[1] "the process may be supposed to take place by very small particles of a somewhat different nature originally existing in or being distributed through the previously homogeneous protoplasm, then collecting at definite places, and appearing as separate masses." It must be noted that this process accords strictly with the principle of "division of labour." In certain vegetable cells the chlorophyll is uniformly disseminated[2], and not collected into chlorophyll bodies. This appears to show that the power of junctioning in a given manner may, in some cases, be localised, in others disseminated.

The disappearance of a nucleus (in Peziza) by its substance being "distributed through that of the protoplasm"[3] seems to point to the same possibility. In some cases the protoplasm of a cell is "tinged by a colouring matter . . . . . which is not present in the cell-sap."[4] It is, therefore, not impossible that this should be the case in Drosera.

The following observation seems to show that in Drosera the crimson colouring matter is not in the cell-sap. Kühne states[5] that when a cell in a Tradescantia-hair dies, the dead protoplasmic network absorbs the purple cell-sap, and becomes in this way stained. Now, in the case of Drosera no such appearance has been observed either by my father or myself. When a cell dies the colour fades away from the crimson cell-contents, and nothing but a dingy mass of granular *débris* remains; this seems to show that the colour is not in the same state as in Tradescantia, but that it is in intimate connection with something living. It should be observed, however, that the colour is in no way essential to the process of aggregation, as it occurs in almost colourless or light-green tentacles.

The general aspect of the phenomena in reference to the two theories above mentioned having been considered, a few of the details will be examined in the same manner.

The physical condition of the masses varies with the stage of aggregation in which they are. The process commences by the cells becoming "slightly cloudy from the for-

[1] 'Handbook,' p. 45.
[2] Sachs, p. 46.
[3] Sachs, p. 11.
[4] Sachs, 'Handbook,' p. 40.
[5] 'Das Protoplasma,' 1864, p. 94.

mation of numberless only just perceptible granules, which rapidly grow larger."[1] Ultimately, when the aggregation becomes intense, and especially when produced by a solution of carbonate of ammonia, the cell contains a single spherical mass or only one or two large spherical ones (fig. 3). These are of a deeper colour and refract light more strongly than the earlier formed masses. The change of colour connected with an alteration in the degree of condensation is shown in fig. 7; the masses here drawn were in process of dissolution; in A we have two dense and dark masses at one extremity of the cell (nearest the gland) and some lighter-coloured ones at the other end; the process of dissolution travels up a tentacle from the base towards the gland, so that in B, which represents the same cell drawn at a later period, we find the masses which were separate, and dark coloured in A, coalesced, and of a lighter tint.

In the early stages of aggregation the masses are extremely motile, and seem to be carried by the currents round and among each other in that peculiar plastic way that one sees in the coloured blood-corpuscles, where currents are set up among them.

In the strongly aggregated condition they become nearly or quite motionless, and seem to be of a fairly dense nature, and on pressing the cover glass they assume the star-like aspect with radiating fissures, described by my father[2] and shown in fig. 5. The change in motility might be due according to my father's views to the crimson protoplasm assuming a passive instead of an active condition; according to the second theory, it might be due to aggregated cell-sap becoming too dense to be influenced by the protoplasm which supplies the motive power. When tentacles die in an aggregated state, the masses usually become turbid instead of remaining clear, and gradually break down into a granular *débris*, which fills up a large part of the cell. The fact that the death of the cell affects the condition of the masses is more in favour of their being of a protoplasmic nature than of their being mere mechanical aggregates. Oil globules, for instance, are not affected in this way by the death of the cells in which they are contained. On the other hand, turbidity is a characteristic sign of the death of such protoplasmic structures as are transparent during life, *e.g.*, cornea corpuscles.

Effects of Reagents.—These effects are most advantageously studied on dense and firmly aggregated masses which

[1] 'Insectivorous Plants,' p. 45.
[2] 'Insectiv. Pl.,' p. 47.

withstand the action of strong reagents. In order that the tints produced by the reactions may be perceptible, it is essential that very pale leaves should be selected; the dwarfed plants growing hidden in moss answer this purpose well.

The masses are not soluble in absolute alcohol or in a mixture of turpentine and creasote; either of these would dissolve oils; the aggregated masses are therefore not purely oleaginous.

They are not coloured blue by iodide or by Schultze's solution, and therefore are probably not amylaceous. A pale aggregated tentacle placed in a solution of caustic potash assumes a yellow tint; the masses not being dissolved; on removing the superfluous alkali and adding strong nitric acid the masses assume the orange-yellow colour of xanthoprotein. If a tentacle is placed in syrup and afterwards in strong sulphuric acid, the masses become reddish brown.

The aggregated masses (in a tentacle killed by osmic acid) are stained yellow by iodine. The three last-mentioned reactions are given by Sachs[1] as tests for protoplasm. But we must not therefore conclude that the aggregated masses consist of protoplasm; for Sachs adds that the above-mentioned reactions are characteristic of "true albuminoids as caseine, fibrine, albumen. We are at least justified in assuming that the aggregated masses consist of albuminoid substances.

A tentacle was placed in strong solution of hæmatoxylin (which acted, I presume, as a poison); the staining fluid was absorbed at one of the minute papillæ and the aggregated masses in the neighbouring cells became brightly tinted with it. The power of absorbing colouring matter is mentioned by Sachs as a characteristic of dead protoplasm and other albuminoid bodies.

The action of osmic acid on the tentacles was not satisfactorily made out. The aggregated masses of tentacles placed in ¼ per cent. solution of this acid, are intensely blackened and are fairly well preserved from the degeneration into granular *débris* which is the usual result of the death of an aggregated cell. But in some cases osmic acid seems to alter the appearances of the aggregated masses before the death of the cell is caused. Thus in one instance I mounted in water a tentacle in which there were fine ropelike masses of aggregated matter. I then irrigated the preparation with about three drops of ¼ per cent. osmic acid; it was chiefly absorbed at the cut-off end of the tentacle, where it blackened the aggregated masses; but in cells further removed from the cut surface a distinct effect was produced although

[1] 'Handbook,' p. 40.

the aggregation masses were not blackened; the change was conspicuous and consisted in the transformation of the ropes of aggregation-matter into chains of delicate spheres, reminding one of rows of dewdrops on a spider's web;[1] this change occurred very generally throughout the tentacle. The same kind of formation of minute spheres has been observed in non-aggregated cells exposed to the action of osmic acid. In a leaf allowed to remain during the night in half a watchglass of water to which a few drops of $\frac{1}{4}$ per cent. osmic acid had been added, presented in some cells a similar formation of spheres, while a neighbouring cell was absolutely uninjured and contained *changing* aggregation masses. It appears from this that an extremely weak solution of osmic acid is sufficient to alter the appearance of the masses, for the amount absorbed by a tentacle of which *any* cells remain alive must be very small.

Relation of the aggregated masses to the flowing Protoplasm.—In a normal cell before aggregation has commenced the streaming protoplasm does not appear as colourless bands, but as lines of moving granules imbedded in a slightly refracting substance; when, however, the process has well begun, the currents show out clearly as a moving network of colourless granular substance. During the process of dissolution we find cells in which the aggregated masses are nearly dissolved, but which are still divided by narrow divisions into a few large masses, nearly filling up the whole cavity of the cell; and in the narrow spaces between the masses currents of protoplasm may sometimes be observed. Again, in the state of intense activity of movement which is produced by subjecting an aggregated tentacle to certain temperatures, one observes small masses of crimson matter forced by the currents to pass between larger masses. The network of flowing protoplasm is continually changing its form, so that it is possible that it may help to mould into shape or divide the aggregated masses. This possibility is obviously favorable to the theory that the changes in the aggregated masses are impressed on them from without. Nevertheless, it is not destructive to my father's hypothesis, for a plastic substance such as protoplasm would naturally be affected by a rapid current like that which streams in the cells of Drosera.

In *strongly* aggregated cells the current is invisible or

[1] This appearance is possibly similar to the "chains of small globular masses" ('Insectiv. Plants,' p. 207) produced by the action of a strong solution of cobra poison, which, however, is not a poison to Drosera, but merely an intense stimulant.

almost absolutely so; its disappearance is ascribed by my father[1] to the loss of the granules which ordinarily make its movement perceptible, and which, as he believes, are absorbed by the aggregated masses. But although the flowing protoplasm may be invisible, evidence of its existence may be found in the free particles which can be seen occasionally travelling round the cell. Moreover a protoplasmic network can sometimes be detected in a strongly aggregated cell after death. In one case an aggregated tentacle was mounted in water and cemented with dammar varnish,[2] with a view to watching the process of dissolution; this tentacle died before the process began, and Fig. 3 is a drawing of one of its cells. In consequence of death the protoplasmic network became visible, passing, as shown in the figure, from one chlorophyll body to the next, like the processes of connective-tissue-corpuscles, and just as the current does in many living cells (fig. 8). I have also seen the protoplasmic network in specimens killed in osmic acid and left in absolute alcohol for a day or two; they were then transferred to glycerine by Strasburger's method of adding a few drops of the latter to a quantity of alcohol, and allowing it to evaporate. In this way I clearly saw cells in which strongly aggregated masses coexisted with a protoplasmic network. These networks are more delicate than the currents seen in the living cell, I suppose owing to the shrinking caused by the alcohol.

Altogether the impression left on the mind by a study of the protoplasmic currents is that they are not capable of producing the changes that occur in the aggregated masses. This impression is not one that can be tested, for the question is obviously one of degree; a sufficiently abundant protoplasmic network containing small aggregated masses in its meshes *might* be able to produce changes in them similar to those of aggregation. There is another peculiarity of the aggregated masses which seems to shows that they are not passive condensations of cell sap, namely, that they frequently contain large vacuoles apparently filled with colourless, or very slightly tinted, fluid; a cell in which some of the cells are in this condition is shown in fig. 2. In many instances a single mass contains many vacuoles, giving it a far more complex aspect than that of the simple example of vacuolation here given. But what is of importance is, that in the alterations

[1] 'Insectiv. Plants,' p. 48.

[2] This otherwise useful varnish does not answer the above purpose, as it almost always runs under the cover glass after a time. Kühne recommends mastich dissolved in chloroform.

which the arrangement and appearance of the vacuoles undergo give us distinct evidence of *internal* change occurring in the aggregated masses, which is quite inconsistent with the view that their changes of form are impressed from without. On the other hand, both the presence of vacuoles, and the fact of internal changes occurring, are perfectly consistent with the view that the aggregated masses are protoplasm. An observation of my father's points to the same conclusion. He describes[1] how whilst one mass was rapidly increasing and another in the same cell rapidly decreasing, he "was able to detect a connecting thread of extreme tenuity, which evidently served as the channel of communication between the two."

It may be worth while here to sugest a third view combining in a certain sense my father's and the opposing theory; but it is more justly to be described as a modification of my father's view. It might be possible that a condensation of the colouring matter of the cell-sap should form the basis of the aggregated masses, while the power of movement would be supplied by particles of protoplasm separated from the flowing network. In favour of this view might be adduced the observations of Sachs[2] on the hairs of Cucurbita, and those of Kühne[3] on those of Tradescantia. Both of these observers saw small portions of protoplasm separate from the network under the influence of heat in one case and cold in the other, and undergo amœboid changes of form while floating freely in the cell-sap. Now, if such amœboid masses were to permeate the precipitated cell-sap, spontaneously moving crimson masses would be the result.

This view must not be dismissed at once. But the fact that an abundant protoplasmic current may be seen flowing, while numerous aggregative masses are actively changing their shape, seems to show that it is not the true explanation. It must be remembered that quantity of aggregated matter is very considerable; the granular *débris* produced by the death of an aggregated cell almost fills the whole cavity. Moreover, the motive power seems to be intimately distributed throughout the aggregation masses, for very minute threads undergo changes of form. If, then, this motive power (intimately distributed through a large quantity of matter) is supplied by the actual deduction of protoplasm from the flowing network, it is difficult to see how enough of the latter can be left to produce the strong currents which certainly coexist with active aggregation.

[1] 'Insectiv. Pl.,' p. 42.
[2] 'Physiologie Végétale,' p. 74.
[3] 'Das Protoplasma,' p. 103.

A few observations were made on the effects of different temperatures; but on testing the thermometer of my warm stage the graduation was found to be incorrect. I hope to repeat my experiments and also make some observations on electrical stimulation.

In conclusion, a phenomenon connected with aggregation will be touched upon. My father observed that in *Drosera* and in certain other plants such as *Erica tetralix* immersion in solution of carbonate of ammonia causes the chlorophyll bodies to collect into heaps, and, what is more remarkable, these heaps are converted into masses by the coalescence of the bodies. I have unfortunately been unable to attend to this subject and have only cursorily observed this coalescence; but I have frequently noticed the chlorophyll bodies in the cells of a *Drosera* tentacle collected into heaps, as shown in fig. 4. The subject is no doubt worthy of investigation. It is curious that a solution of carbonate of ammonia which is so potent in producing aggregation of the crimson matter in the cells of *Drosera* should also cause a somewhat similar movement in the chlorophyll bodies; and that this aggregation of chlorophyll should occur in other plants than *Drosera*. Sachs[1] describes the changes in position undergone by chlorophyll grains in various unfavorable external circumstances; "for instance, in small fragments of tissue, when respiration is defective, turgidity diminished, temperature too low, the cells too old, or—what is of most interest here—where light is cut off for a considerable time." Under these circumstances the chlorophyll bodies collect together in certain positions. Sachs considers it certain that they possess no power of independent movement, and that their change in position is due to the action of the colourless protoplasm in which they are embedded.

If this holds good with the chlorophyll bodies which collect together under the influence of carbonate of ammonia, the case might be adduced in favour of the view that the crimson aggregated masses are in like manner passive and only moved by protoplasm external to themselves.

Finally, I believe that a fair consideration of the arguments for and against my father's view, that the aggregated masses consist of protoplasm will incline the inquirer to accept it in preference to any opposing theory. I believe moreover that in this way the physiological significance of the process of aggregation is more likely to be justly determined.

[1] 'Handbook,' p. 672.

Remarks *on the* Shell-gland *of* Cyclas *and the* Planula *of* Limnæus. By E. Ray Lankester, M.A., F.R.S. With Plate XXIV.

Controversy is one of the most wasteful ways in which a man can employ himself with pen and paper. I do not propose, therefore, to enter into a verbal dispute with the zoologists who have recently expressed opinions at variance with the results which I have published relative to the developmental phenomena of Mollusca; but I am anxious to take an opportunity of briefly reasserting the chief features of those results; since in late publications, partly by deliberate misrepresentation (Fol), and partly by ignorance (Jherings), they have been made to appear different in point of date, and of actual significance from what they really are.

My observations, commenced in 1871, (Pisidium, Limax, and Limnæus, 1871; Aplysia, Pleurobranchidium, Polycera, Tergipes, Sepia, Loligo, 1871-72; Neritina, 1873; Loligo (bis), Limnæus (bis), Paludina, 1874) led me to certain results of a general character besides those which had reference to this or that species only, and the most important of these were briefly indicated in a paper in the 'Annals of Natural History,' February, 1873. I briefly described, in that paper, the shell-gland of Aplysia and of Pisidium, and introduced the term "shell-gland," which has since been adopted by German writers as "Schalendrüse." I also described, for the first time, the formation of an invaginate Gastrula, *with closure of the orifice of invagination* in several Nudibranchs and in Pisidium; thus extending Kowalewsky's observation to the Mollusca, he having previously mentioned the invaginate Gastrula as occurring in the development of the Heteropod Atlanta. This publication was anterior by some months to that of Professor Ganin, of Warsaw, who in 1873 gave an account of the development of Cyclas and of Limnæus, differing very greatly from my own.

In the first month of 1874, before I left England for a second sojourn at Naples, the MS. and figures forming the memoir published in 1875 were deposited with the Royal Society. On returning to England in July, 1874, I resumed the study of the development of Limnæus, and published in this Journal, October, 1874, a paper which gave an outline of the results embodied in my larger memoir ('Phil. Trans.,' 1875), besides the results obtained from Limnæus.

The facts which I observed in Limnæus in every way confirmed and strengthened the views which I had already expressed in the paper communicated to the Royal Society before leaving England.

Some (and but a few) of the points in the earlier of these two papers, additional to those noted in the paper of 1873, are—1st. The correspondence (as first indicated by Selenka in Purpura, and by Kowalewsky in Oligochæta) of the invaginate and circumcrete forms of Gastrula. 2nd. The continued proliferous activity of the large endodermal cleavage-spheres after enclosure by the smaller circumcrescent spheres in the development of Aplysia, and of the corresponding cells in Pisidium. 3rd. The origin of the cephalic nerve-ganglia from ectodermal cells within the area of the velum. 4th. The mode of formation and evanescence of the shell-gland. 5th. The rectal peduncle or pedicle of invagination in Pisidium.

In the paper on Limnæus I was able to adduce facts which strengthened my views as to the activity of the endodermal cells subsequent to their inclusion within the ectoderm; to confirm my account of the rectal pedicle of *Pisidium* by the discovery of an identical structure in *Limnæus;* to show that what Lereboullet had taken for the mouth of *Limnæus* was its blastopore (the name which I have given to the orifice of invagination, by means of which the endodermal cells become enclosed in the ectodermal in so many animals) which closes, in most cases, at an early period of development, and that what he had taken for an "anal cone" is my shell-gland (as I had previously pointed out, 'Proc. Roy. Soc.,' 1874). I also showed that the Pulmonata are not, as had been supposed, devoid of a ciliated velum in the larval state, and that the velum is not even reduced to a rudimentary condition, as had been supposed, but is well developed and prominent at an early stage of development. Further, I pointed out the existence of the "shell-gland" in various Mollusca, namely, in Pisidium, Aplysia, Neritina, Paludina, Limnæus, Terebratula, and Loxosoma, and discussed its relationship to the open pit in the mantle which I discovered in the embryo Cephalopoda.

These and some other points in my publications relative to Molluscan development have been variously received by subsequent writers. A Genevese, by name Fol, with whom it was my misfortune to spend two hours at Messina, in May, 1874, has made them the ground of a gross personal attack in M. Lacaze Duthier's 'Journal of Zoology.' He has, however, been compelled, in the face of evidence sub-

mitted by me to the readers of that journal, publicly to withdraw the charges of theft made by him, though he has not had the manliness to apologise for his attempt to injure a scientific *confrère*. On the contrary, he continues to exhibit an unworthy spite, which I need not notice in detail.

Mr. Hermann Jhering has published two papers on the development of Mollusca,[1] in which he has criticised my observations upon the development of Pisidium. He is good enough at the outset of his remarks relative to myself to allude to the 'staunenswerthe Unkenntniss' and 'zugellose Phantasie des Autors.' This rough sort of abuse though unmannerly is not such as to call for a reply. We are not accustomed to it in this country in scientific literature, but those of us who read German periodicals know that it is more frequently met with in those publications, and is merely the fashionable way of saying that you disagree with a brother zoologist. Mr. Jhering, however, goes on further with his criticism, and entirely misrepresents my views. He says ('Jenaische Zeitsch.,' vol. ix, p. 311), "Ray-Lankester regards the byssus-gland of the Acephala in so far as its secretion is concerned, as not only the first rudiment of the shell-ligament of the mussel shells, but he declares that it exists also in Limnæus under the shell in relation with the mantle—ground enough for him to pronounce straightway the internal shell of Limax to be homologous with it (the byssus-gland), his shell-gland." He proceeds further to declare that I have apparently never heard of the byssus-gland. Not for the benefit of Mr. Jhering, who appears to write with the humorous intention of making as much confusion as possible, but for the sake of those who might accept what he has said in the 'Jenaische Zeitschrift' and in the 'Zeitschr. für Zoologie' seriously, I will point out that there is not the remotest ground for supposing that I have mistaken the byssus-gland for the shell-gland in Pisidium. When I discovered the shell-gland in Pisidium *it was unknown in that or any other mollusc*. I was perfectly well acquainted with Leydig's and Schmidt's observations on the development of Cyclas, and I looked for the byssus-gland in its proper place. So far as I followed Pisidium (up to the development of four gill-filaments only), no byssus-gland is developed. In Cyclas it does make its appearance before that stage, as do also the oto-cysts, which certainly do not appear so early in Pisidium. I did not

[1] 1, "Ueber die Entwickelungsgeschichte von Helix," 'Jenaische Zeitschrift,' vol. ix, p. 299; and 2, 'Ueber die Ontogenie von Cyclas" (devoid of illustrations), 'Zeitschrift für Wiss. Zoologie,' Bd. xxvi, p. 414.

overlook these structures, but I *looked carefully* for them and determined their *absence*. Mr. Jhering appears not to have reflected on the enormous improbability of an observer discovering a new organ, such as the shell-gland (subsequently observed and recognised by other observers) through mistaking another organ for it.

In his second paper Mr. Jhering explains more at length his interpretation of my published drawings of Pisidium development. He declares them to be "sehr skizzenhaft," and accordingly assumes that they are grossly inaccurate. I am ready to admit that some of them may be found wanting in the finer histological details, but they are not sketches: they are careful drawings made from specimens under the microscope, and some of them by aid of the *camera lucida*. They were made five years ago, but I cannot entertain the supposition that they are erroneous in such points as Mr. Jhering suggests. Mr. Jhering declares that what I have shown (as I think) to be the primitive invagination to form the hypoblast is the real "shell-gland," that my shell-gland is the byssus-gland, and that what I have called the rectal pedicle or pedicle of invagination is the œsophagus. This looks to me very much like wanton contradiction. There are some limits to the probable not to say possible errors of a practised observer. When such sweeping condemnation is attempted as that which Mr. Jhering has ventured upon, one looks for evidence in the form of new observations illustrated by careful drawings. This Mr. Jhering does not think it necessary to offer; and in the absence of such "material for discussion," I can only say that I do not think that he has brought forward any fact which affects the accuracy of my statements. Mr. Jhering cites the observations of Professor Ganin, of Warsaw, which were published some months subsequently to my first statements with regard to Pisidium. At present I have not heard that Professor Ganin has published any figures in illustration of his observations. As far as I can gather from the abstract in Schwalbe and Nitsche's 'Jahresbericht' Professor Ganin's observations coincide in some points with my own, whilst in others we differ considerably. But I am unable to fully apprehend his views as to either Cyclas or Limnæus in the absence of illustrative drawings.

[Since the above was written I have taken occasion to examine the embryos of Cyclas, at Oxford, and I am able to confirm most emphatically, from the examination of that form, my previous statements as to Pisidium. The genus Pisidium is separated by systematists from Cyclas mainly on

the ground that in the former the siphonal tube is externally single, whilst in the latter it is bifid. In the accompanying plate (Plate XXIV, figs. 1 and 2) I have given a drawing of two embryos of *Cyclas cornea* (two out of many observed by me at the end of April this year) which is a true copy of the appearances seen with a power of 400 diameters, minute histological details being omitted. From this it will be seen that *both 'shell-gland' and 'byssus-gland'* are present in the young Cyclas; both are perfectly defined and obvious. I am led to infer from this what is probable enough from analogy, namely, that *Pisidium pusillum* does not develop a byssus-gland at all, or if at all at a late period, whilst *Cyclas cornea* develops a byssus-gland at an early period, soon after the shell-gland has made its first appearance.]

Mr. Carl Rabl has a paper also in the ninth volume of the 'Jenaische Zeitschrift' entitled "Ueber die Ontogenie der Süsswasser Pulmonaten." Mr. Rabl at the conclusion of his memoir compares his observations with my own on Limnæus and whilst expressing his disagreement with some of my conclusions does so in a simple and straightforward way which appears to me perfectly legitimate and rational. He has adopted in his paper Lereboullet's view as to the anal nature of the cone-like shell-gland and maintains this view in spite of my demonstration that it is the shell-gland. Probably by this time he would admit that he has been in error on this matter. He also maintains that there is a condition of the Limnæus embryo when it consists of a number of equiformal cells arranged in the form of a hollow sac (blasto-sphæra). This, he says, I have overlooked. On the other hand I maintain that such a phase does not occur, and that Rabl has made a mistake in following Lereboullet as to this matter as he has in the matter of the 'Anal-kegel.' Mr. Rabl expresses regret that I had not used the method of transverse sections in my study of Limnæus development and states that I should then have arrived at other conclusions than those which I have adopted, concerning the origin of the hypoblast. I am not aware, however, that Mr. Rabl or any other embryologist has succeeded in cutting sections of embryos so small as are the earlier stages of Limnæus. The figures which he himself gives are diagrams based upon 'optical sections.' Mr. Rabl also considers that I have overlooked the otocysts and the canals of Stiebel, and the pedal ganglion. To this I must reply that in the stages most carefully examined by me (stages equivalent to those preceding his figure 25) these structures with the exception of Stiebel's

canals (the urnieren) were not present. The fact that they are figured by Mr. Rabl has led me to re-examine these stages in specimens obtained from the same locality as those which I previously studied.

I find that the 'nephridia' or primitive kidneys, known as Stirbel's canals, are present in the position described by Rabl. They escaped my attention owing to the fact that I was engaged with other points, viz. the shell-gland, intestine and velum. The otocysts and pedal ganglia do not appear until later.

It is a pleasure to me to have finally to draw attention to the recent publications of Professor Fleming, 'Wiener Sitzungsber.,' Bd. 71, 1875, and 'Zeitschr. fur Wiss. Zool.,' 1875) relative to Molluscan embryology—since he has been led to accept the accuracy of my observations and finds in them the explanation of some difficulties connected with the interpretation of his own results obtained in the study of Unio and Anodon.

Formation of the Gastrula in Limnæus.—In my paper on the embryology of Limnæus I pointed out the difficulty which I had experienced in tracing the precise mode of formation of the invaginate planula or gastrula from the morula of that animal.

I have endeavoured to overcome that difficulty since, but cannot consider what I now have to submit as final.

The interpretation which I have given of figs. 4, 5, 6, pl. xvi, of my memoir in vol. xiv of this Journal, is erroneous, and to a certain extent the drawings are incorrect. Fig. 4 of that plate is more correctly represented in fig. 6 of the present paper—and fig. 5 of that plate by fig. 5 of the present.

I am not able to agree with those who maintain that there is a distinct blastula phase in the development of Limnæus in which the cells are equi-formal. I find that continuously the cells of the antiklastic pole (that is to say those opposite to the pole at which the directive corpuscles escape) exhibit a larger size and a greater abundance of brown granular food-material than do those of the klastic pole.

After the stage represented by fig. 4 of the present plate, the cells of the klastic hemisphere commence to flatten out and to recede from those of the antiklastic hemisphere, leaving a central space between the two groups—which is filled with liquid and is traversed by tapering processes of the attenuated cells of the klastic pole. These no doubt are the first indications of the filaments connecting endoderm and ectoderm which are figured in pl. xvi, fig. 14, and pl. xvii, fig. 19, of my former paper. The klastic hemisphere

in consequence of its dilatation by liquid has a much paler appearance than has the solid mass of granular cells grouped in the opposite hemisphere. It is at first difficult in many specimens to make out the cells which form the wall of the vesicular region—and in my former paper I made the mistake of regarding this part of the embryo as consisting of four big cells. The four big cells which I thus misplaced really belong to the opposite—the anti-klastic hemisphere. They are seen in pl. xvi, fig. 5, of my former paper (where the directive corpuscle R is quite erroneously introduced), and are also seen in fig. 5 of the present plate.

In fig. 8, an embyro, with its more diaphanous vesicular hemisphere and its darker granular hemisphere, is represented as seen from the surface. In fig. 7 the same embryo is focussed to the median plane and the cavity traversed by the processes of the paler cells is seen.

The exact number and arrangement of the cells in the dark antiklastic hemisphere is difficult to ascertain. The four which are seen by a view of the antiklastic pole undoubtedly become invaginate and give rise by division to the primitive enteron. They take up this position partly in consequence of the circumcrescence of the surrounding cells which are seen to the number of eight in figure 5 of the accompanying plate encroaching upon them. Partly, however, their own growth and division is in such a direction as to form a basin-like area with its concavity facing the antiklastic pole (see figs. 7 and 10 of pl. xvi of my former paper). Thus the mode of formation of the diploblastic Limnæus is intermediate between epibolé and embolé. The widely open blastopore with its shallow basin-like archenteron (or gastrula-stomach) now narrows rapidly and assumes the elongated slit-like form represented in fig. 9.

This *elongated* form of the blastopore is important in connection with the question of the persistence of the blastopore as either anus or mouth. Where the blastopore actually persists as, or is identical in position with, the anus, it has a *circular* margin which narrows to a minute passage as in the Echinoderms and Paludina (see on the Planula of Paludina, Lankester, vol. xv of this Journal, p. 159). In Limnæus, and in some other Gasteropods the blastopore is elongated. Its posterior end (marked *Rect.* in fig. 9 of the present plate) closes up, and thus is formed the rectal peduncle which I previously figured, and of the existence of which I do not feel any doubt in spite of the failure by Rabl and others to detect it. I have preparations (prepared with osmic acid and glycerine) which show it clearly. The anterior end of

the blastopore marked *or* in figure 9 corresponds with the position of the permanent mouth, but before the widening of the permanent pharynx is formed it becomes exceedingly small, and I do not feel sure as to whether it closes up entirely as I previously maintained or whether it remains throughout (as Lereboullet believed), being at one period an exceedingly small aperture. In any case we cannot say that there is a direct conversion of the blastopore into the mouth, but rather a coincidence of the stomodæal invagination (invagination to form the Vorderdarm) with one end of the elongated blastopore. The middle third of the blastopore corresponds strangely enough with the position subsequently occupied by the foot.

The proctodæum or anal invagination does not occur till very late in Limnæus. The rectal peduncle is blind as in Pisidium and Cyclas; it must not be confounded with the Hinterdarm or Proctodæum, but is a part of the Urdarm or Archenteron.

Note *on* Mihakowics' New Method *of* Imbedding. By H. N. Moseley, M.A. Oxon., Naturalist on H.M.S. Challenger.

In a late number of the 'Archiv für Mikroskopische Anatomie' (II Bd., 3es Hft., p. 586) a new method of imbedding animal tissues for the purpose of preparing fine sections of them is described by Mihakowics, who used the method for the investigation of the vertebrate embryos. I have employed Mihakowics' method, with some very slight modifications, in the preparation of sections of decalcified corals, especially the Stylasteridæ, with great success. In all methods hitherto employed the imbedding substance has had to be removed by solutions from the interstices of the section of tissue before the section could be mounted and rendered transparent for examination. In the present method a substance is used which does not require removal, but is maintained *in situ* without diminishing the transparency of the object. The substance holds the delicate parts together, and maintains them in their relative positions in the section, whereas in methods where soap, &c., are used, the parts separated by section swim away most

provokingly all over the slide on the removal of the imbedding substance by benzine, oil of cloves, &c. The imbedding substance made use of by Mihakowics consists of a jelly composed of equal parts, by weight, of glycerine and gelatine. The glycerine is heated in a warm bath, and the gelatine dissolved in it. An extremely tenacious jelly is the result. I found this jelly too tough for use with corals, and I added a larger proportion of glycerine. The tenacity of the jelly can be modified to any extent according to the nature of the substance to be cut, just as the consistence of the oil and wax mixtures is varied to suit the tissue to be imbedded; but it must be remembered that the less tenacious the jelly, the more it will shrink when placed in absolute alcohol. Mihakowics stained his hardened embryos, then placed them in absolute alcohol, and, just before putting them in the imbedding substance, placed them for a minute in water in order to avoid great shrinking, which otherwise occurs. I hardened my corals in chromic acid, absolute alcohol, or osmic acid, decalcified them in weak hydrochloric acid, and then soaked them in glycerine, previously staining those hardened in absolute alcohol. The corals were transferred directly from the glycerine to the warm jelly. I found that the jelly more thoroughly penetrated the spongy tissues when they were thus previously soaked in glycerine. The imbedding jelly is kept just fluid over a water bath, and the small masses of tissue are macerated in it for one or two hours until all their interstices are thoroughly permeated by the warm jelly. In the case of corals, which, by the removal of the supporting calcareous skeleton become excessively spongy, it would be well, no doubt, to place the vessel in use under an air pump for a short time. There was, unfortunately, no air pump available on board H.M.S. Challenger. When the tissues have been well soaked in the jelly they are transferred, together with a portion of jelly, to small cavities scooped out in small blocks of liver which has been hardened in ordinary alcohol, and are arranged in the proper position for cutting, and the cavities filled up with the jelly. When the jelly has set, which condition most rapidly ensues, the blocks of liver are placed in absolute alcohol, and allowed to remain there two or three days. The jelly becomes hard, white, and opaque, and the liver hardens, and shrinking around it holds it firmly. The liver and hardened jelly are now cut into sections in the ordinary way with a razor, wetted with absolute alcohol, and the sections are treated with glycerine upon the slides. The opaque jelly becomes in a few minutes perfectly transparent, and is

almost invisible in the preparations. Some difficulty is experienced in transferring the tissue and warm jelly to the cavity in the liver before the jelly sets or becomes stringy. A teaspoon, previously heated in boiling water, is a most convenient instrument for the purpose. This method supplies a want which has long been felt. It is eminently adapted, for the preparation of sections of structures contain an abundant calcareous skeleton, and which are apt to collapse on the removal of this by acids, such as Echinoderms, corals, &c., but no doubt it will yield equally good results in such structures as Corti's organ, insects' eyes, &c., where it is desirable to retain *in situ* in a section a number of elements which have no organic connection or a very slight one with one another.

NOTES AND MEMORANDA.

Rev. E. O'Meara on Irish Diatomaceæ. — Those of the readers of this Journal interested in the Diatomaceæ, forming as they do a considerable section of its supporters, will be glad to have their attention directed to the recent work of that able and practised observer in that wide field, the Rev. Eugene O'Meara, whose contributions have frequently ere now added to the value of these pages. Under the seemingly somewhat insufficient title of "Report on the Irish Diatomaceæ," part i, that author has published, in the 'Proceedings of the Royal Irish Academy,' vol. ii, ser. ii, No. 4, October, 1875, what is really by no means a mere catalogue (as might possibly be supposed from the title) of the forms found in Ireland, but it in reality amounts to a comprehensive Monograph (or will be when the second part appears) of the whole of the Irish species in the Diatomaceæ, accompanied by critical remarks on the views of his predecessors as to identification and specific specialities, and by a reference to the works of foreign contemporary authors. Whilst the wide experience and protracted researches of the author, in various parts of the island, extending over several years, have enabled him vastly to extend the number of the older-known species recorded as Irish in Smith's 'Synopsis' (1853 and 1856), he has likewise, from time to time, introduced to the notice of microscopists many new species in this family, which, so far as they are Irish, are here recorded. It is a matter of regret that means were not at command to have illustrated the work more copiously, by giving figures of all the species, or at least of those (a great number truly) not to be found in Smith's 'Synopsis.' If there is something to be desired as regards the execution of the nevertheless numerous figures representative of the genera, or of, in the author's view, their sub-sections, given on nine crowded plates, as compared with that of former illustrations from Mr. O'Meara's hand,

which have appeared in this Journal, they are still most valuable as the work of an observer so thoroughly conversant with the distinctive features and characteristics requisite to be especially pourtrayed in relation to individual forms.

The author precedes the Classification and Descriptive Enumeration of the species by a short Introduction, in which he speaks of the Motion, the Structure of the Cell, the Reproduction, the Classification, and the Distribution of the Diatomaceæ.

As to Classification, the author prefers partially to follow Heiberg ('De Danske Diatomeer,' Copenhagen, 1863) rather than Pfitzer ('Ueber Bau und Entwickelung der Bacillariaceen,' Bonn, 1871, in Hanstein's 'Botanische Abhandlungen'); but, opposed to Heiberg, he prefers to keep together the forms with cuneate outline as a special group (in place of associating, as does Heiberg Meridion, and Asterionella with Fragiliarieæ, as "Fragiliarieæ Cuneatæ;" Podosphenia with Striatelleæ, as "Striatelleæ Cuneatæ;" Gomphonema and Cocconeis with Naviculeæ, as "Naviculeæ Cuneatæ;" and he also prefers still to attach more importance than does Heiberg to the circumstance as to whether the frustules are free, or attached by a gelatinous cushion, or by a more or less elongate stipes, or concatenated in chains, or enveloped in more or less definite mucous tubular fronds: he rather recognises these varied modes of growth as of generic value, which is perhaps in some cases questionable. But to decide between the diverse opinions of the two authors would require a very lengthened and repeated study of the organisms in the living state, from period to period and season to season—a field of observation which, on the whole, seems too much neglected by diatomists. The advantage, to beginners at least, of the author's work, containing as it does such a great mass of the accumulated experience of an able observer, would have been enhanced by analytical keys of the groups and genera, to form, as it were, finger-posts for the guidance of strangers in this very extensive—possibly to them wholly unknown—territory, albeit the adept could find his way at once. Such, it is understood, Mr. O'Meara will prepare and append to the second forthcoming part of his work, which is expected to be ready to appear, through the same channel, in about a year, and no doubt diatomists will look forward to it with impatience. The mode of breaking up of the long genus Navicula (with which Mr. O'Meara combines Pinnularia), and the grouping of the various species, betokens the long and careful study of the author. It is questionable, upon coming to the individual

forms themselves, if the descriptions are sufficiently minute and exhaustive, at least in the absence of figures of all the species, for the purpose of mutual comparison and contrast; for though the differences in many cases be in themselves seemingly slight, they are doubtless, in reality, none the less actual and intrinsic, therefore the words employed to verbally delineate those slight but characteristic differences could hardly too closely follow their minutiæ. Localities for nearly all the species described are given—hardly requisite, indeed, for a goodly number of "common" forms—but, on the other hand, of great value as regards the "rarities." No doubt the work in question will be critically examined by those observers in this country and abroad who have made the Diatomaceæ the object of special study; and whether they should find therein views to concur in or dissent from, this record of painstaking research and careful study should be in the hands of each specialist in this branch of microscopy.

Action of Sulphate of Thorium on the Blood Corpuscles.—At a meeting of the San Francisco Microscopical Society, Dr. Blake, of San Francisco, exhibited some blood obtained from a rabbit after the introduction of sulphate of Thorium into the blood vessels. Although the quantity used was only a grain, yet the physical properties of the blood had been very much altered, the blood corpuscles having entirely lost their natural form and presenting indented outline with numerous small highly refractory dots at the circumference. This marked influence on the blood corpuscles tends to explain the highly poisonous character of the salt, death taking place in two minutes after the introduction of the above minute quantity into the circulation. It is also in accordance with the law which connects the physiological action of substances with their isomorphous relations and atomic weights, thorium having the highest atomic weight (115) of any of the members of the aluminium group which its physiological reactions show it to be isomorphous.—(*American Journal of Microscopy*, May 1876.)

Fusisporium Solani and its Resting Spores.—Mr. Worthington Smith writes in the 'Gardeners' Chronicle,' p. 656:—*Fusisporium Solani* is a fungus which very commonly occurs on diseased potatoes in company with *Peronospora infestans*. One is as destructive to the potato as the other, and Mr. Berkeley, writing of the former in 1857, describes it as a second enemy of the potato, "equally destructive with the Peronospora, and, according as the two are separate or com-

bined, different appearances arise. In some cases," continues Mr. Berkeley, "it produces an extreme degree of hardness, inducing a condition like that of the mummified silkworms. Sometimes, on the contrary, it causes rapid and loathsome decay, especially when in company with the Peronospora." Like the latter, it suddenly appears on the potato plant, carries on its work of destruction, and vanishes.

Till now I believe the resting condition of *Fusisporium Solani* has never been described. In my attempt to work out the history of *Peronospora infestans*, the undoubted resting-spores of the Fusiporium came to light in the following manner:—A quantity of badly infected potato leaves were selected and isolated last July with the view of watching the Peronospora. As the presumed oospores of the latter gradually appeared, there also appeared much smaller bodies, which also went to rest; these were so similar in size and appearance to antheridia or dead zoospores, that they were thought to belong to one or the other. When I recently placed some of the presumed oospores of Peronospora in pure water to promote germination, all the smaller bodies at once burst, and in the short space of six hours developed into perfect plants of *Fusisporium Solani*. In size the spores measure about the $\frac{1}{2500}$ of an inch in diameter; they are palish brown in colour, with a very finely muricated outer coat, and a light central nucleus. The Fusisporium is frequently produced close to the resting-spore, and I have observed the direct germination and production of the Fusisporium in innumerable instances. How these resting-spores arose last year I am not certain, but it is not improbable that they may be a different condition of the aërial fruit broken up into four parts.

Pythium Equiseti.—Mr. Worthington Smith writes in the 'Gardeners' Chronicle,' p. 656:

Great interest is just now attached to this curious parasite, and hitherto it has not been recorded as British. Dr. Sadebeck, of Berlin, described the plant last year as a new species of Pythium, parasitic upon Equisetum arvense. It bears a considerable resemblance to the bodies discovered last year, and referred by me to the secondary condition of the Potato fungus. It ultimately appeared that Dr. Sadebeck also last year found a similar parasite infesting and destroying living Potato plants near Coblenz, and at the time he referred the Equisetum and Potato parasites to the same fungus, and on seeing my micro-photographs he doubtfully threw out the suggestion that all three fungi might possibly prove to be the same with each other.

On these insufficient grounds a report was spread in this country that the organisms described by me were the same with Dr. Sadebeck's Pythium Equiseti, and the 'Journal of Botany' for March last stated, in reference to Pythium Equiseti, that it had "lately been attempted to connect this fungus with the oospores of Peronospora infestans." Dr. Sadebeck can hardly be said to have made such an attempt, for in a very kind letter that he wrote me on March 23rd last he said the presumed identity was a mere "supposition," thrown out in a preliminary paper, that he was without experiments from which to form a definite conclusion, and that he had not been able to infect the Potato plant artificially with the Pythium.

Dr. Sadebeck's excellent paper, and the evident strong external resemblance of his newly discovered plant to mine, made me extremely desirous of seeing the Berlin plant, but on writing to Dr. Sadebeck to this effect he replied that he had no specimens. It therefore only remained to look out for the parasite here, and I was fortunate enough to enlist the good services of Mr. B. D. Jackson, F.L.S., who sent me some capital specimens of Equisetum arvense from Snodland, Kent, on April 25th. The first piece of Equisetum I examined under the microscope displayed the presence of fungus spawn ramifying amongst the tissues; so, from experience gained of the habits of some of the lower fungi, I half submerged the specimens of Equisetum and kept them covered up in a dark place. In ten days the Equisetum plants were dotted inside and out with gelatinous patches, and every patch was a mass of Pythium Equiseti. Though bearing a strong resemblance to the early condition of the bodies found by me in the Chiswick Potatoes, yet Pythium Equiseti is clearly not the same. Mr. Berkeley, who has seen both plants, writes me that he considers them "decidedly different." I have been unable to infect Potato plant with the Pythium or the Equisetum with my Potato oospores. My experiments, therefore, agree with the results obtained by Dr. Sadebeck, and the two parasites may be considered different.

* * * * *

A few lines should here be given regarding the parasite found by Dr. Sadebeck on Potatoes. First of all it may be said that De Bary, who also, it seems, met with a new parasite on Potatoes, could not make it artificially take possession of the living Potato plant, and principally for this reason he refers the parasite, not to Peronospora, but to Pythium. Dr. *Sadebeck*, however, found his fungus in possession of the

living plant, for he says that "in the first days of July, 1875, he saw at Metternich, not far from Coblenz, a potato field which, to all appearance, was affected with the murrain; a closer examination, however, showed that the signs of disease were traceable almost entirely to Pythium Equiseti. The anticipated Peronospora was not found in any of the plants examined; on the contrary, the Pythium was discovered in a great number of plants, and in all parts of the plants." It would seem not improbable, therefore, that Dr. Sadebeck really met with the oospores of Peronospora infestans, if one may judge from the effects and habit of the parasite, no specimens being preserved or experiments carried out.

PROCEEDINGS OF SOCIETIES.

Dublin Microscopical Club.

20*th January*, 1876.

On the structure and presumed transformation of Irish Limestone.—Professor Hull, F.R.S., exhibited a thin slice of the limestone of Haulbowline Island, Co. Cork, in confirmation of his view that the rock has undergone a complete transformation since its original deposition: assuming that the Carboniferous Limestone of Ireland, where unaltered, always showed either to the eye or under the microscope, organic structure, it was argued that when found with the crystalline structure only, the original rock had been subsequently replaced. In the case of the Haulbowline limestone and of the red marble from the adjoining quarries of Little Island, it was considered that a nearly complete transformation had taken place, by which the planes of bedding were nearly obliterated and numerous veins of calc-spar had been introduced. Professor Hull believed this process to have taken place after those terrestrial disturbances by which the Carboniferous Rocks of the South of Ireland were forced into numerous flexures, and he gave an account of the manner in which he considered that, through the agency of water charged with carbonic acid gas, the original limestone had been dissolved, and from time to time redeposited in the crystalline form. The thin slices showed clearly the planes of cleavage of the rhombohedron traversing the rock in various directions, sometimes in slightly curved lines, and along with this was a total absence of organic structure.

Exhibition, new to Ireland, of the new parasitic Rhodosperm—Choreocolax Polysiphoniæ, Reinsch.—Dr. McNab showed for the first time in Ireland the newly discovered parasitic rhodosperm, *Choreocolax Polysiphoniæ*, Reinsch, found by him on some examples of that alga taken at Salthill, Co. Dublin. These form little cellular hemispherical prominences on the host, when older, lobed; in at least one instance, the "rootlike" immersed portion of the parasite could be traced down into the substance of the host Polysiphonia. These only lately discovered coloured parasites formed a subject of great interest, and much credit was due to Professor Reinsch in having directed attention to so many

new forms of algæ of strictly parasitic nature, so long overlooked, though possibly many of them may be widely enough dispersed.

Some rare Rhizopodous forms exhibited, with a new species of Quadrula, Schulze.—Mr. Archer had on the table some seemingly rare Rhizopodous forms, though the great difficulty of finding in a moment objects so minute and so readily evading observation prevented his being able to exhibit them individually. Amongst these was *Plagiophrys sacciformis*, Hertwig et Lesser, a form of *Pseudochlamys patella*, Clap. et Lachm., as well as *Quadrula symmetrica* (Wallich) Eilhard Schulze, and he was further able to show a new form appertaining to the latter genus. Mr. Archer must altogether concur with Professor Schulze in the propriety of making a genus distinct from Difflugia, of which Dr. Wallich's form *D. symmetrica* was the type, and, in illustration, Mr. Archer was able to exhibit at same time a specimen of that very marked form. The new form which he had now the pleasure to show was distinguished from *Q. symmetrica* by being smaller, quite without any "neck," the opening irregular and without any rim-like border; the square or oblong, occasionally triangular, "plates" of the test much more irregular and varying in size; it showed also now and again a few spines comparable to those of *Euglypha compressa*, but much fewer. The "plates" were of much more variable size than in *Quadrula symmetrica*, very large and very small side by side. They often showed a square inner space which was either much thinner than the *frame-like* outer part, or this space represented possibly a square opening occupying a greater or less proportion of the area of the plate, sometimes, indeed, reduced to a minute dot; often indeed, absent, and the plates like those of the other form. Thus this form, apart from the want of any projecting border to the mouth of the test, might be said to bear a relation to *Q. symmetrica*, somewhat comparable to that of *Euglypha globosa* to *Euglypha alveolata*. Mr. Archer hoped to prepare a more enlarged description and a figure of this form, which he would call *Q. irregularis*, for some future occasion.—Mr. Archer further presented a preparation in Beale's Carmine fluid of the curious Amœba form, *plus* a cluster of finger-like posterior appendages=Ouramœba, Leidy, which, however, did not cause any contraction of the processes, a fact, so far capable of being urged by Professor Leidy in favour of his views; but, on the other hand, the gathering abounded with specimens of the ordinary character, that is, without the faintest evidence of any linear processes—simple *Amœba villosa* (*princeps*),—but, the appendages apart, quite identical with the so-called Ouramœba.

Blood-cells from a leukhæmic patient exhibited.—Mr. B. W. Richardson exhibited a mounting of blood-cells taken from a leukhæmic patient; the white cells were largely increased in number and varied in size; the resemblance of many of them to pus-cells was very striking.

Exhibition of section of Spine of Strongylocentrotus lividus, in

illustration of effects of injury to Spines of Echini.—Mr. Mackintosh communicated some observations on the result of injury and repair to the spines of Echini. He found that the general result was the development of the reticulated structure at the expense of the solid pieces, and this might go so far as to convert all the secondary part of the spine into network, leaving no trace of the solid pillars, but usually these latter persisted, though very much reduced in size. A section of the altered spine of *Strongylocentrotus lividus* was exhibited, in which the central part was occupied by a hollow surrounded by the usual central network, which is about double the normal amount, and sends out thick somewhat triangular "spokes" between the considerably reduced pieces.

Sporochisma mirabile (on dead Hollyleaves) exhibited.—Mr. Greenwood Pim exhibited *Sporochisma mirabile*, B. and Br. A remarkable torulaceous fungus, described as parasitic on beech-branches. It occurred in this case on dead holly twigs at Hollybrook near Bray. It consists of simple flocci with a tough inarticulate exterior membrane which breaks up into quadriarticulate spores. There is a rather strong superficial resemblance (though, of course, nothing more) to some Scytonematous Algæ.

Multinucleated Cells from epithelium of Frog's stomach.—Dr. Purser showed a mounting of epithelial cells from a frog's stomach, presenting the remarkable peculiarity of possessing from one to several (sometimes as many as six) nuclei, a condition which had never before come under his observation; the animal was a healthy female.

Lejeunia calyptrifolia exhibited.—Dr. Moore exhibited under a low power some pretty examples of the rare and minute *Lejeunia calyptrifolia*. This ordinarily grows on other Jungermannieæ, but the present examples were formed on the bare rock.

Closterium obtusum, Bréb., *new to Ireland, exhibited.*—Mr. Archer showed for the first time what was undoubtedly *Closterium obtusum*, Bréb., thus helping to complete the series of forms possibly capable of being regarded as a distinct genus between Closterium, Penium and Cylindrocystus, of which he had exhibited two new forms at the November meeting. He also presented an example of *Spirotænia obscura* which seemed to show that the view he had suggested as regards the internal structure was correct, and that that form was not truly congeneric with *Spirotænia condensata*, *Sp. muscicola*, *Sp. parvula*, etc., but was more related to the series of forms in question.

Cosmarium Schliephackianum, Nordst., *new to Ireland, exhibited with its Zygospores.*—Mr. Archer had on the table conjugated examples of *Cosmarium Schliephackianum* (Grun.), Nordst. just taken in the "Rocky Valley" near Bray; this gathering he had had under observation, and had come to the conclusion that it was well-marked and new, when next morning's post brought (for which best thanks to the author) Herr Nordstedt's recent paper on

Arctic Desmidieæ delineating the very form in question. (Öfversigt af Kongl. Vetensk.-Akad. Förhandl., 1875. No. 6, t. vii, f. 15.) Its oblong, rectangular zygospores call to mind those of *Penium Mooreanum*, Archer, but they want the nipple-like prolongations at the angles, as in that species, but like them they often appeared *twisted*, even so much so that the planes of the opposite ends of the zygospore might be at right angles to one another.

17th February, 1876.

Lejeunia echinata exhibited.—Dr. Moore showed *Lejeunia echinata*, and pointed out the mode of fructification in this species.

New species of Tænia exhibited.—Dr. Macalister showed a new species of Tænia which be named *T. Hyracis*, and of which he would hereafter publish a description.

Exhibition of sections of Spine of Phyllacanthus annulifera.—Mr. Mackintosh showed cross sections of the spines of *Phyllacanthus annulifera*, A. Agaz., which presented the usual cidaroid characters.

Exhibition of Crystals of Xanthine Sulphate.—Mr. B. Wills Richardson exhibited a very perfect stellate mass formed of Crystals of xanthine sulphate mounted in Canada-balsam. He, at the same time, reminded the members present that in December, 1872, he exhibited crystals of the hydrochlorate, the nitrate, and also the sulphate of xanthine. An illustrated description of the hydrochlorate appeared not long afterwards in the 'Quarterly Journal of Micr. Sci.' to which he referred those who might require further information about them. The specimens of the sulphate exhibited on that occasion were not so perfect, however, as he could have wished, having been more or less injured in the attempt to clean them for mounting. Having originally but little more than a couple of grains of xanthine (from human urine) to act upon, of which only about a grain remained after the formation of the crystals he had described, he determined to try if he could prepare some more crystals of the sulphate and, if successful, to mount them without any attempt to clean them. Accordingly he placed about a quarter to half a grain of the xanthine on an ordinary slide, and then added to it a little pure sulphuric acid. The slide was placed in a cabinet, secured from dust. Crystallization slowly took place which ended in the formation of the very perfect stellate mass now under the microscope. It was not until the expiration of two years that the specimen was sufficiently dry to allow of his mounting it in Canada-balsam. During the slow process of drying, if it could be called such, he occasionally removed superfluous acid with small pieces of bibulous paper. The crystalline mass was globular and studded with long projecting crystals, some of which were facetted and appeared to belong to the triclinohedric system, but of this he did not wish to speak too positively. In the polariscope,

with the assistance of selenite, the polariscopic effect was very beautiful.

Strychnia Sulphocyanide Crystals exhibited.—Mr. R. J. Moss exhibited characteristic crystals of strychnia sulphocyanide prepared from strychnine, which had been extracted from the contents of the stomach of a Newfoundland dog, seven days after death. Mr. Moss stated that with the aid of the microscope, he had applied most conclusive chemical tests to a minute portion of the strychnia, thus being able to reserve the greater portion of it for further examination in case of necessity—an important consideration in toxicological investigations.

Stauroneis platystoma, Ehrenb., *exhibited.*—Rev. E. O'Meara exhibited a form of Stauroneis which he considered identical with *Stauroneis platystoma*, Kütz. The figures given by Kützing, "Bacill." t. III., f. 58, and by Rabenhorst "Süssw. Diat." t. IX., f. 2, are very deficient as respects details of striation, but the external outline, so different from all other forms of this genus, renders identification certain. The striation is very fine, linear and slightly radiate. The only place in which specimens of this species were found by Mr. O'Meara was Lough Mask, near Tourmakeady, Co. Mayo.

A new Minute Fibrous Sponge.—Dr. E. Perceval Wright exhibited a minute sponge, the frame-work of which was chiefly formed of horny or fibrous material, scattered throughout which were a few acerate silicious spicules. The general appearance reminded one of the pentacrinoid stage of Antedon, before the tentacular arms were developed. The cup-shaped portion crowned the summit of a beautifully fashioned stem, which was attached by an expanded disc to the fronds of several species of algæ. The cup-shaped portion was more or less spherical, slightly flattened at the top and presenting but a single osculum. The stem called to mind a string of Amphitetras-frustules. Whilst it was easy to perceive the affinities of such a sponge, no already formed genus seemed fitted to receive it. The total height of the largest specimen known was from two to three mm. and an apology might be needed to form a genus or species for such an "atom." If but a solitary specimen had turned up, it might have been admired and forgotten, but whilst working up some Algæ from Tasmania, again and again either perfect forms or the beheaded stems of this little sponge appeared, showing a perfect sameness of form and structure, almost too of size. Remembering how persistent very minute forms are amongst the calcareous sponges and that such remain sufficiently constant to be regarded as species, one gets sufficient courage to introduce this as the smallest of all known fibrosilicious sponges, to be described and figured hereafter as Kalispongia Archeri.

A new Form of Freshwater Amphistomatous Rhizopod exhibited.—Mr. Archer showed two out of five examples (all he had as yet seen) of a new Rhizopodous form taken at "Tooles Rocks," Co. Wicklow. This, *but* that it was absolutely devoid of any incrus-

tation of foreign particles, possessing, as it did, two opposite stomata at either end of an elliptic test, for emission of rhizopodous pseudopodia, would fall under his own genus Amphitrema. Drs. Hertwig and Lesser, in their very thorough treatise on freshwater Rhizopoda[1] proceed throughout on the principle of separating otherwise kindred forms into distinct (collateral) genera, dependent on the character as to the test being a pure secretion-product or being incrusted with foreign mineral particles or diatomaceous frustules and fragments, &c.—a principle which Mr. Archer, so far as he could see, had felt altogether called upon to approve. But, in respect to the present new form, coming away from the incrusted Amphitrema across the line so drawn, the only other established genus of Amphistomatous Rhizopods was Dr. Barker's Diplophrys. Still no one, viewing the two or, including *Diplophrys Archeri*, three organisms, could arrive at any other conclusion than that the new one now exhibited was really by far more closely and naturally allied to *Amphitrema Wrightianum* than to *Diplophrys Archeri*—in fact, to refer the new form to Diplophrys would surely be artificial in the highest degree, keeping Amphitrema in view. But if not to be referred to the latter genus on account of the test being quite bare of foreign particles, in fact, absolutely smooth and glossy, there would be nothing for it but a new genus bearing a relationship to Amphitrema comparable to that of Plagiophrys, Clap. et Lachm., to Pleurophrys, Clap. et Lachm. In the new form the stomata were very sharply marked openings, with a slightly developed rim projecting somewhat inwards and with a perfectly "clean" edge; test smooth and glossy, quite without any markings and of a yellowish colour, of a somewhat thick and rigid character. Mr. Archer exhibited side by side with the new form some examples of *Amphitrema Wrightianum*, which he had had for some time by him treated with Beale's carmine fluid, showing the nucleus half way between the two openings, the test however had become in itself very pellucid and its brownish colour gone. This seemingly decidedly rare species he had taken only on some half dozen occasions, but the stations wide apart—some from Glen-ma-lur, Co. Wicklow, some from Co. Westmeath, some from Co. Galway, some from Co. Kerry, and these (as well as the new form) from "Tooles Rocks," Co. Wicklow. It is seemingly remarkable that as yet *Amphitrema Wrightianum* has not been found on the continent, notwithstanding the recent very active researches by various observers in this field, and it was perhaps a curious coincidence that Mr. Archer should likewise be the first to encounter a second, or rather counting Diplophrys, a third amphistomatous form, whilst the latter (*D. Archeri*, which has now a companion in *D. stercorea*, Cienk.—an unsavoury propinquity in which to find one's self!) most puzzling

[1] Hertwig and Lesser: "Ueber Rhizopoden und denselben nahestehende Organismen" in Schultze's 'Archiv f. Mikrosk. Anatomie,' Bd. x (1874).

form has been found in many places on the Continent, and, though already well "threshed," remains still no little puzzle.

23*rd March*, 1876.

Blodgettia confervoides, Harvey, *from a new station (Bermuda), and remarks on the possible parasitic nature of the presumed internal strings of "spores."*—Dr. E. Perceval Wright exhibited specimens of *Blodgettia confervoides*, Harvey, from Bermuda. As is well known Dr. Harvey described and figured this alga in his 'Nereis Boreali-Americana' as occurring at Key West and referred it to the family Valonieæ. Although one of the most wonderful of the Algæ this plant does not appear to have been studied or investigated during the twenty years that have elapsed since it was forwarded to Dr. Harvey by Dr. Blodgett, from Florida. On the authority of Mr. Farlow ('Proc. Amer. Acad. of Arts and Sciences,' vol. x, 1875) M. Bornet had suggested that the spores (?) are parasitic unicellular Algæ, and, if so, then the plant itself will be undoubtedly a Cladophora—either, *Cl. cæspitosa*, as at first imagined by Harvey, or near *Cl. prolifera*, as suggested by Farlow. The occurrence of the plant at Bermuda is not without interest, believing, as Dr. Wright did, that M. Bornet's surmise was right—it was noteworthy that it occurred in the cells of the same species of Cladophora. It was also of importance to recollect that the Blodgettia—adopting the name in a new sense—was to be met with only in the very oldest cells. There were numerous difficulties in the way of making out the minute details of structure from long-dried specimens, and it is to be hoped that further light may be thrown on the affinities of this form by some botanist residing near either of its now known localities. Some superficial resemblance might be observed between the moniliform string of spores as figured by Harvey and the spores of *Synchytrium anomalum* as figured by Schroeter, but it would be dangerous without much further study to even hint that there might be an affinity between the two.

Crystals of Sulphate of Indigo exhibited.—Mr. B. Wills Richardson exhibited a mounting of Crystals of Sulphate of Indigo prepared with sulphuric acid and pure indigo, the latter kindly given to him by Prof. Emerson Reynolds. Some of the crystals were tinted of a delicate blue colour and the forms of some were stellate, many of the stellæ having a striking resemblance to one of the varieties of stellæ of triple phosphate found in human urine. All the crystals were polariscopic, the stellæ resembling the triple phosphate apparently the least so. Mr. Richardson observed that he had some difficulty in getting the acid and indigo to form the crystalline compound and after several attempts he succeeded by the cautious application of heat. When sufficiently dry the crystals were mounted in dammar solution.

Section of Spine of Phyllacanthus baculosa exhibited.—Mr.

H. W. Mackintosh exhibited a cross section of the spine of *Phyllacanthus baculosa*; the only difference that he could detect —beyond mere colour and size distinctions—between it and the spines of *P. annulifera* was in the character of the central network, which was circular and sharply defined, and had comparatively slight solid rods in the latter, whilst in *P. baculosa* it was irregular in outline and much less distinctly marked off from the surrounding tissue and had thick solid rods.

Puccinia Smyrnii, Corda, *on same leaf with an Æcidium, exhibited.*—Mr. Greenwood Pim showed *Puccinia Smyrnii*, Corda, in the Uredo-condition accompanied on the same leaf by the spermogonia of (apparently) an Æcidium, and suggested the query as to whether their occurrence together had any bearing on the statements that Puccinia and Æcidium are different stages of the same plant. This species which is described as rare in Cooke's Manual appears to be abundant in many parts of the Co. Dublin, wherever *Smyrnium olusatrum* occurs.

A form of Coscinodiscus, probably C. apiculatus, Ehrenb., *exhibited.*—Rev. Eugene O'Meara exhibited a form of Coscinodiscus found by him in the stomach of ascidians dredged in the neighbourhood of Kinsale. Upon first inspection it might be regarded as identical with *Coscinodiscus perforatus*, Ehrenb., but a closer examination presented characters very distinct from those of the species named. The areoles surrounding the hyaline boss are much smaller and more numerous, and are generally more decidedly hexagonal than in *C. perforatus* and more closely arranged and moreover they increase in size towards the margin. Mr. O'Meara considered this form to be likely identical with *Coscinodiscus apiculatus*, Ehrenb., 'Microgeologie,' t. xvii. f. 43.

Plagiophrys spherica, Clap. et Lachm., *exhibited, and remarks on the so-called Gromia granulata*, Schulze, *and on the form referred to by latter author (erroneously), as Microgromia socialis* (Archer), Hertwig et Lesser. — Mr. Archer showed living examples of the Monothalamatous Rhizopod he had referred to *Plagiophrys spherica*, Clap. et Lachm., a form which however Hertwig and Lesser seemed inclined to suppose not to be precisely that species. Be that as it may it was surely at least a form of the genus Plagiophrys, but strange to say this or one very like it seemed to be referred to the genus Gromia, (Dujardin), by Prof. Eilhard Schulze under the name of *Gromia granulata* (Schultze's 'Archiv f. mikr. Anat.,' Bd. xi, p. 117, t. vi, f., 5,6), from which genus it would appear altogether distinct in appearance and habit and behaviour. If Mr. Archer had erred (and if so it was *not very far*) in placing his *Gromia socialis* to that genus (now made the type of a new one by Hertwig and Lesser, under the name of Microgromia), surely still more had Prof. Schulze erred in placing a form like that now exhibited thereto. The allusion to *Microgromia socialis* (Archer), Hertwig and Lesser, suggsted to Mr. Archer to mention that he thought there could

be no doubt but that the Rhizopod shown on Prof. Schulze's plate and referred by him to that species appears again to be quite a different thing ("social" in habit, no doubt) and to appertain more probably to Plagiophrys and not to Gromia (Microgromia). Mr. Archer had long since discovered his own error in referring the densely crowded state of *Microg. socialis* to a distinct genus; he hoped however for an opportunity ere long to furnish a résumé of the recent matter on these rhizopods in general, and submit the reasons which seemed to him to sustain the opinion as to the foregoing forms here enunciated.

A very small form of Cosmarium Holmiense, Lundell, *exhibited*.—Mr. Archer drew attention to a very small form of *Cosmarium Holmiense*, Lundell, not probably more than one half the linear dimensions of the ordinary form. So great a difference in the dimensions of examples in a single species of the Desmidieæ, unlike Diatomaceæ, was rare to note, for ordinarily in the former the variations in dimensions oscillated between certain rather limited extremes. These examples (probably owing to being taken at the "dead" period of the year) showed the cell-contents very starchy in appearance and the radiate (Penium-like) arrangement of the chlorophyll-mass to be seen in the larger form of *Cosmarium Holmiense*, Lundell, was not evident. In this small form the general contour in all respects agreed with that of the larger, except that the little undulations on the lateral margins, as they approach the somewhat drawn-out upper angles, were rather less pronounced. The ordinary form seems in Ireland to be very scarce, yet widely spread, as Mr. Archer had taken it now at Bray Head, "Tooles Rocks" and "Rocky Valley" (Co. Wicklow), Townland of Clashnasmut (Co. Tipperary); the present (very small) examples were from the "Rocky Valley," near Bray, where also the larger form occurs.

Medical Microscopical Society.

Friday, 19*th May*, 1876.

Dr. F. Payne, President, in the Chair.

Scirrhosis of Liver in a Child.—Mr. Needham communicated the particulars of a case, and showed specimens both of the liver and other organs. The child, æt. six years, was admitted as a patient in the North Eastern Hospital, Hackney Road, on June 23rd, 1875. Her antecedents were good and the child's health had never suffered till lately, when she was observed to be losing flesh, and six days before admission her abdomen began to swell. She had always been well taken care of and had never taken spirits in any form. In July the abdomen was tapped, but soon again refilled; 42 oz. of fluid were removed. The child rallied and returned home, but was readmitted in December, 1875, much worse, suffering from attacks of epistaxis and hæmatemesis; and in January, 1876, she died in convulsions after having vomited a quantity of coffee-ground material. Post-mortem examination showed the liver to be "hobnailed," indurated and weighing 19½ oz. It was uniformly straw-coloured, no puckering of Glisson's capsule, and a section appeared fleshy, intersected with bands of a tough material; spleen firm, 6 oz. in weight. Other organs healthy, but anæmic. Stomach contained food and streaks of blood, but the source of the hæmatemesis could not be found.

A section of the liver showed with the microscope liver structure cut off into island glands of connective tissue, in which were elongated cells that readily were stained with carmine; and besides these, dotted about, and easily stained, were very many round cells, like white blood-corpuscles, to which they seemed more related than to the long cells above mentioned. The liver cells were often confused with this new growth and were undergoing fatty degeneration in some places. Glisson's capsule was healthy and had taken no part in the process. The kidneys showed proliferation of connective tissue, as did also the spleen: and between the gastric tubes in the stomach was much of the small cell-growth, such as was seen in the liver.

The various organs had been prepared with chromate of ammonium and also with spirit, and had been stained in various ways, but the same appearances always presented themselves. In between the muscular fibres of the heart, and in the brain, especially about the blood-vessels, the same small cell-growth could be well seen.

The President had never heard of a case where the changes were so universal. Similar conditions in the stomach and kidney

had been before described, but never, as far as he was aware, in the heart. It would be extremely interesting if cirrhosis of the liver could be made a general disease. He had also seen a case of cirrhosis of the liver at the age of four years.

Dr. Pritchard suggested the presence of the small cells in the various organs pointed only to some irritation of the blood-vessels, and to no particular diseased condition.

The President inquired if the cells were found equally around arteries and veins; for if so it was against simple inflammation being the cause.

Mr. Needham, in reply, stated that in another case of cirrhosis that he had examined he had found all the changes here described. The proliferation of small cells were equally around the arteries and veins.

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MEMOIRS.

Résumé *of* Recent Contributions *to our* Knowledge *of* "Freshwater Rhizopoda." Part II. Heliozoa (continued). Compiled by Wm. Archer. (With Plates XXI and XXII.)

In accordance with the views held by Hertwig and Lesser they include in the group Skeletophora several Heliozoan species in which I myself do not recognise the presence of such hard parts as could properly admit of being designated as "Skeleton;" such, for instance, are *Astrodisculus*, Greeff, *Astrococcus*, Greeff, *Chondropus*, Greeff, and *Heterophrys*, mihi. Now, as I cannot therefore, at least as yet, coincide with those writers, I am prevented in this résumé from following them in placing such forms in the group Skeletophora. But although the series of forms in question are indeed as yet regarded by myself as *without* "*skeleton*" in the strict sense, still on the other hand they are manifestly in advance of the three forms already referred to—Actinophrys and Actinosphærium, with (if admissible as distinct from Actinophrys) Ciliophrys (Cienkowski)—in the possession of the distinct and considerably differentiated outer region, in a sense, constituting "skeleton" for Hertwig and Lesser. This outer coat without spicules or spines clearly seems to place them apart on the one hand from the naked forms and on the other from the truly skeleton-bearing. One or other of two plans suggests itself to me to avoid this difficulty—either we might call Actinophrys (with Ciliophrys) and Actinosphærium *Askeleta nuda* and the group in question *Askeleta chlamydophora*, or we might allow the former to remain Askeleta (*they* are incontrovertibly *destitute* of *any* skeleton) and the latter to stand as an intermediate group equivalent to the former as well as to the Skeletophora proper (equally incontrovertibly *provided with* skeleton) under the name Chlamydophora.

But, further, it seems likely that the establishment of yet another group may be called for to embrace those seemingly Heliozoan forms which cover themselves round with a more or less deep and more or less loose or compact, but apparently

always rudely applied, stratum of *foreign* fragmentary objects, such as arenaceous particles and diatomaceous frustules, &c. Such would seem to be Lithocolla (Eilh. Schulze), and possibly Elaeorhanis (Greeff).

I propose, then, next to take up the newly recorded forms falling under such a group intermediate between the naked and truly Skeletophorous forms, and possessing an outer stratum which must at least be a true secretion-product, yet without spines or spicules. The reasons which induce me to disagree in this regard with Hertwig and Lesser and to the proposal of such a group will present themselves on considering the forms as they turn up.

Heliozoa Chlamydophora.

I propose to group under this designation necessarily only such Rhizopoda as are of a true homaxial nature, and, like the Askeleta, indeed, *without* spicules or spines or any solid or hard skeleton, but *with* a distinct highly differentiated soft and more or less mobile external envelope, either granular or of a striate appearance, or seemingly quite homogeneous in nature. The form named by Eilhard Schulze *Heterophrys varians*—the same thing, I believe, as Greeff's *Heliophrys variabilis*—should not, I conceive, be included here, owing to its not being strictly homaxial, but must, I fancy, be *ad interim* relegated to Hertwig and Lesser's incongruous group along with Leptophrys, &c.

Astrodisculus, Greeff.[1]

This genus, so far as the forms appertaining to it are approximately known, seems to offer the most marked exemplar of the chlamydophorous askeletous type. As the descriptions, so far as they are given by Greeff, have been already reproduced in the pages of this Journal (l. c.), it would be unnecessary to repeat them in detail. It is, however, desirable to point out, as bearing on the question under consideration, that the outer region appears to be of a soft and homogeneous nature, hyaline, and sharply bounded at the periphery. It is true that Greeff regarded this outer zone in his *A. minutus* as probably siliceous and extremely minutely perforate, to allow the passage outwards of the pseudopodia—if that were *truly* the case it would be "desmothoracous." He makes, indeed, the important observation, if confirmed, that it was not altered by application of sulphuric acid. Still, he goes on to

[1] Greeff, "Ueber Radiolarien-artige Rhizopoden des süssen Wassers," in Schultze's 'Archiv f. Mikr. Anat.,' Bd. v, p. 496, 'Quarterly Journ. Micr. Sci.,' vol. x, p. 113 et seq.

say that through this "porous" coat not only the pseudopodia proceed outwards, but also more or less large corpuscles are able to pass, so that he was inclined to assume that the coat possessed "certain larger openings or was in part formed of a soft permeable organic substance." He further on says that the extremely fine pseudopodia can, as in the other species, with readiness be followed, through what he now calls the "outer protoplasma-ring," up to and into the body. He records a "central globular structure" (doubtless truly "nucleus"), though in *A. ruber* (see fig. 3) he could find no "central vesicle or nuclear structure."

Hertwig and Lesser describe under the name *Hyalolampe exigua* a form which appears mainly to be distinguished from *H. fenestrata*, Greeff = *Pompholyxophrys punicea*, Archer (see hereafter) only by its greater minuteness and the greater delicacy of the "skeleton," the spherules of which are superimposed in more numerous layers. Their contours can only be discerned under a very high amplification. The authors mention that frequently one pre-eminently large pigment-granule was often present, sharply contoured, globular, and of a ruby-red.

The authors think it highly probable that this form may be identical with that named *Astrodisculus ruber* by Greeff (see Pl. XXI, fig. 3), which, except as regards the outer "skeleton," really seems to present much resemblance; Greeff describes the outer envelope in his form as presenting a slightly granular but otherwise hyaline "sarcode" stratum, which, as mentioned, he regarded as outwardly porous and silicious, the pseudopodia making their way out through the supposed pores. Having regard to the great difficulty of recognising the composition of this outer skeleton in Hertwig and Lesser's form, which under insufficient amplification might appear homogeneous, it is quite conceivable that these authors may be correct in their supposition of the identity of Greeff's form with theirs; but, could this be proven, should not Greeff's *specific* name (just as applicable) hold good? (or, rather, should not the form in question stand as *Pompholyxophrys rubra*, Greeff?)

Hertwig and Lesser, indeed, are inclined to go further, and conjecture that all the forms referred to his genus *Astrodisculus*, differing, as they do, only in *colour* (a somewhat variable characteristic) may be referable to their *H. exigua*. I am myself inclined to doubt the propriety of this assumption, although forms seemingly referable to Astrodisculus being, in my own experience, amongst the very greatest rarities, and, when found, so scanty, I have been unable to submit them to any experiment. Although Hertwig and

Lesser are doubtless correct in referring the coloured globules in Greeff's forms merely to large pigment-corpuscles, not as Greeff does, to "central capsules," still the outer envelope does appear different to that of other Heliozoan forms. In the very few forms I have been able to see this part of the organism certainly did appear to be quite homogeneous, structureless, and sharply contoured externally. Once I saw a form in which there were *two*, mutually sharply marked-off, homogeneous and seemingly soft concentric layers, externally quite smooth throughout, through which the delicate pseudopodia projected.

In fact, such a (single-layered) Heliozoan has not escaped Hertwig and Lesser themselves, though they have not been able to work it out. These authors, indeed (and I would regard the record by so accurate observers of such a form as a confirmation of the views here sought to be established), only give a brief mention thereof in a note (l. c., p. 224). They say that "in fact Heliozoa do occur whose superficies is enveloped by a homogeneous delicate stratum with the aspect of but a light also, its outer limit recognised only with difficulty." They figure it on t. iv, fig. 7 (l. c.). Now, such a nucleated, homaxial rhizopod, with its homogeneous sharply bounded outer coat, as they allude to and depict, is precisely my own idea of an "Astrodisculus."

If there be such fine openings in a silicious envelope through which crude food could be incepted and the larger granules expelled, as stated by Greeff, certainly in the very few examples seemingly referable here which I have seen no such characteristic could be detected and nothing of such an appearance is depicted by Greeff himself; it appears to me that during such process the outer envelope must just simply temporarily give way. It is certainly a pity that the forms seemingly referable here are so extremely rare in their occurrence that an opportunity so much needed to submit them to experiment but very rarely offers itself.

Now, I fancy such a form may, as it were, represent a Pompholyxophrys (= Hyalolampe) *without* the peripheral spherules, but *with* some such an external stratum, perhaps, indeed, of more dense nature than such as one may suppose to hold together the globules of the latter genus, and thus differing, on the other hand, from Heterophrys in the homogeneous (not granular), non-mobile, and non-fimbriate character of the outer envelope; for one may suppose that the spherules of Pompholyxophrys, just as the acicular spicules of Raphidiophrys, would hardly of themselves, without the presence of some connecting medium, of however great tenuity,

remain in mutual coherence, and could hardly but often get removed and lost.

I fancy the so-called *Astrodisculus radians*, Greeff, cannot belong to the genus, but it more likely appertains to Acanthocystis.

Astrococcus rufus, Greeff[1] (Pl. XXI, fig. 2),

is described as possessing a "reddish-brown globular body, surrounded by a hyaline, colourless, cortical stratum, in and on which dark shiny granules move about vigorously. The colour of the inner substance is caused by the presence of numerous minute pigment-granules. Upon pressure larger hyaline granules are ejected from the interior, similar to the pale granules of Acanthocystis, only smaller." The author could not discover "a single larger nuclear structure." He describes the pseudopodia as "fine and linear, emanating from the reddish-brown body, passing outwards through the hyaline cortex, and mostly shorter than the diameter of the body." Along the pseudopodia minute granules could be seen in active movement up and down.

These are the whole of the particulars as regards this form communicated by the author, called in his memoir *Astrococcus rufus*, but in the explanation of the plate referred to as *Astrococcus rubescens*. Granting the validity of the author's own genus Astrodisculus, one is somewhat at a loss to see why he makes a new genus for the present form (enclosed by a "hyaline colourless cortical stratum") unless he had become satisfied that in Astrodisculus the outer stratum was truly siliceous (which still I fancy appears doubtful, as I have mentioned), and that that region here was not so. As regards the statement that he did not find a nucleus, there cannot be much doubt, arguing from analogy, but that the carmine solution would have disclosed it.

Heterophrys, Archer.

After giving my own conception of this doubtless necessary and sufficiently well-founded genus,[2] Hertwig and Lesser draw attention to some points in which they are unable to concur with my views as to structure and nature of some of the parts. As regards the presumed absence of a nucleus, I had long since found out my oversight, but in extenuation I must say it is a part of the organization extremely difficult to discern; however, the application of Beale's carmine fluid rarely fails to disclose this body, though having to be allowed to act for

[1] Greeff, 'Schultze's Archiv,' Bd. xi, p. 27, t. ii, f. 19.
[2] 'Quart. Journ. Micros. Sci.,' vol. ix, p. 267; x, p. 107.

some time, it is often sufficiently difficult on a fresh occasion to refind the rhizopod itself amongst the mass of *débris* so frequently unavoidably taken up in one's efforts to alight upon these forms, often so fond, apparently, of lurking commingled therewith in some out-of-the-way ambuscade. Where we as yet really seem to differ is in our conception of the outer region, external to the sharply contoured body-mass giving off the pseudopodia ; this these authors cannot regard as any kind of granular protoplasmic sustance, but as "a skeleton of extraordinary delicacy, so much so that the highest powers allow only an insufficient resolution of its composition." They rightly describe its appearance, separated as it is from the surface of the body by an extremely narrow but distinctly evident clear space, and as if united thereto only by means of the pseudopodia, as that of "a stratum of granules, dots, and fine striæ, from which[1] arise extremely fine spines in a more or less regular radial arrangement. However much at first glance the skeleton gives the impression of a loose and transitory consistence, it still possesses a considerable coherence. We could not perceive, so long as the organism was alive, that it became disintegrated, or that, when dead, any breaking up of the skeleton was induced. The apparently granular stratum can indeed hardly consist of cemented granules, since these could scarcely remain so firmly combined as to form for the spines seated there on a secure basis." They then compare this external (so-called) skeleton-portion to the "spongy" fabric described by Häckel as appertaining to many Radiolarians, only of much greater delicacy ; or again they compare it to a skeleton of spicules felted together after the manner of a sponge fabric.

I confess from all this to see not any reason to materially alter my original conception of this portion of the build-up of the forms falling here ; although the description given by these authors of its optical appearance is unquestionable, the fimbriate border presented by *H. myriopoda* seems to me not to be due to the presence of *spines*. Were they such (like Acanthocystis) they should occasionally (by pressure or otherwise) be capable of being detached ; but they appear to me simply as slender, but somewhat rigid, prolongations of the superficial stratum or outer region of this investing envelope. Still they do not seem comparable to pseudopodia ; they doubtless seem rigid, but scarcely hard, linear processes. In an example of a form found by me in a moor pool not seemingly distinguishable from *H. marina*, Hertwig and Lesser,

[1] (In Heterophrys myriopoda, H. marina).

the application of Beale's fluid caused the contraction, or a kind of coagulation as it were, of this outer region and the disappearance of the fimbriated margin, the processes composing it seemingly contracting into a few mere short, rounded, thickened, stumpy-looking elevations. Now the true spines of several (two or three) examples of *Acanthocystis Pertyana*, which luckily happened to be on the same slide, were not altered at all—in fact it would have been highly surprising if they had been. But further, in the living state it appears to be of, so to say, a soft nature, more or less yielding to certain (restricted), doubtless accidental, changes of figure of the whole organism. In the form which I called *Heterophrys Fockii*,[1] I may admit, as being destitute of the fimbriated margin, possibly not wholly correctly relegated to one and the same genus, this outer region is still more plastic and changeable in contour and even in position.

It has always rather struck me that this cloudy layer of granular, and striate (in *H. Fockii*, of "streaky") appearance, must be considered as analogous to the certainly less solid and less pronounced—might one say more ethereal-looking—envelope sometimes noticeable, more or less involving the spines, and the lower parts of the pseudopodia of *Acanthocystis turfacea*, as referred to hereafter, as well as to the same region in the genus Raphidiophrys.

But to go a step further and take into consideration the interesting form referred to this genus, but seemingly really distinct owing to its not being homaxial and possessing branching pseudopodia and numerous nuclei, called *Heterophrys varians* by Prof. Eilhard Schulze,[2] we have a form in which this outer envelope, which, however, is clear within and granular, but not fimbriate, at the periphery, is for a time normally absent, only ultimately making its appearance. This Schulze (possibly not inaptly) compares to the gelatinous investment exuded by many algæ, rather than to a "skeleton." I have no doubt that this form has occurred to myself, but I had not sufficient material at command to subject it to a study. There can be no doubt, however, but that it is the same as that recorded by Greeff, under the new name of *Heliophrys variabilis*,[3] a *résumé* of whose description will be given hereafter.

As regards the minute peripheral clear space around the central body and the outer envelope in question, we are I

[1] 'Quart. Journ. Micros. Sci.,' n. s., vol. ix, p. 267.

[2] Schulze, "Rhizopodenstudien," Schultze's 'Archiv f. Mikr. Anat.,' Bd. x, p. 386.

[3] Greeff, in 'Schultze's Archiv für Mikrosk. Anat.,' Bd. xi, p. 28.

suppose to presume that Hertwig and Lesser regard it as void of living substance, and possibly retaining only water. This may be so in older specimens, but one must suppose there was once a mutual genetic connection. But I should rather myself suppose this is only a narrow region of the outer envelope of a highly pellucid character.

Heterophrys marina, Hertwig and Lesser.[1] (Pl. XXII, fig. 13.)

As indicated by the specific name, the authors found this form in sea-water; however, a single example of one in which I could see no tangible distinction therefrom, as mentioned, occurred to me in a gathering made from a moor pool, a further evidence, so far as it goes, in favour of those authors' arguments above referred to, and against the presumption that freshwater Heliozoa may be but primitive forms, or even a more lowly group, of Radiolaria. As regards the body-mass in this form, the authors see the differentiation of a homogeneous endo- (fig. 13, *k*) and a granular ectosarc (fig. 13, *r*), to be equally well marked as in Acanthocystis, which can be here all the more readily observed owing to the absence of chlorophyll-granules, the endosarc bearing only colourless rounded granules of varying sizes and sharply contoured crystals belonging to the rhombic system. They did not observe contractile vacuoles, nor did any show themselves in my own (single) example. There is a submedian oval nucleus, with nucleolus, immersed in the endosarc (fig. 13, *n*). The pseudopodia are in length about twice the body-diameter, numerous, and granuliferous.

Coming to the external region, the authors, in accordance with their views, call it "skeleton," and this presents the same fimbriated periphery ("spines," Hertwig and Lesser) as that of *Heterophrys myriopoda*, mihi. See remarks above on this part of the structure, as regards indeed the only example of this form I have met with, and from the freshwater (if, indeed, it can be truly the same). In fact, except for the marine habitat, smaller size, absence of chlorophyll-granules, pale (not slightly brownish tinted) outer region, and comparatively longer pseudopodia, the authors would have felt inclined to refer it to *H. myriopoda*, mihi; the discovery of a *colourless* form in the freshwater, so closely resembling their form, renders it indeed most probable that so it should be. In *Acanthocystis turfacea* the presence of the chlorophyll-granules is not constant, though pale examples are but rarely met with; I have more than once seen speci-

[1] Hertwig and Lesser, loc. cit., p. 213, t. iv, f. 4.

mens one half green, one half colourless, the line of change sharply marked off; this species, too, varies a good deal in size. The same amount of variation in these respects may occur, possibly, in *H. myriopoda*.

Heterophrys spinifera, Hertwig and Lesser.[1] (Fig. 14)

is a freshwater form, distinguished from the preceding by the comparatively greater length of the linear processes ("spines") of the outer envelope, as well as of the pseudopodia. It certainly appears, judging from the figure, to be a distinct form, yet its differences difficult to define, for I venture to suggest the character of the *supposed large interval* between the body-mass and the granular and then fimbriated outer region, as compared to the preceding, may not be founded in fact. If we compare it in this regard with *Heterophrys varians*, Schulze,=*Heliophrys variabilis*, Greeff, we see an outer envelope of considerable depth, devoid, it is true, of any of those capillary processes (spines, Hertwig and Lesser) at its own periphery, and that, towards the periphery of the inner sarcode body (except in one of Greeff's figures), it is quite as hyaline as Hertwig and Lesser's figure shows for *H. spinifera*. I do not think it can be maintained that in *H. varians* the outer (living) substance is restricted to the granuliferous concentric distant border, with vacancy, or only water, intervening between it and the body-mass; certainly in examples I once myself took I should never have fancied the pellucid interval, permeated here and there by pseudopodia, to be non-living and empty (save of water), in fact, just as little as one would suppose the granular-looking and striate outer portions of the very thick gelatinous envelopes to certain algal forms to be separated from the plant within by a vacant interval, merely because it appears homogeneous and hyaline. In Hertwig and Lesser's figure of their form the granular portion gradually fades off inwardly, without showing any line of demarcation limiting this supposed distant "skeleton" inwardly.

In accordance with their views, however, the authors describe the body of this form as freely poised in the middle of the distant granular stratum, forming a hollow sphere, in which it is sustained in position solely by the pseudopodia. They consider a protoplasmic composition of this "spine-bearing" granular layer as out of the question, as they record it did not yield to sulphuric acid. This, no doubt, is a very important observation; but, on the other hand, I have no

[1] Hertwig and Lesser, loc. cit., p. 215, t. v, f.

doubt of the correctness of my own observations above referred to, as regards the contraction of the so-called spines of *H. marina* (or a small colourless form of *H. myriopoda ?*) and seeming "coagulation" of the outer layer upon lying some time on a slide under "Beale's fluid."

As regards the nature of this outer region in general, I would not contend that it is "protoplasmic" or purely sarcodic like the inner body-mass; it is manifestly of quite a different texture and consistence; but still it is not "hard," nor containing "hard" constituents, like the spines of Acanthocystis, Raphidiophrys, &c., but *comparatively* soft and yielding, very much so in the form *not* externally fimbriated, referred to Heterophrys (perhaps questionably) by me as *H. Fockii*, and in the form, kindred but not properly falling here, *H. varians*, Schulze = *Heliophrys variabilis*, Greeff; in the outer envelope of "typical" species of Heterophrys (*H. myriopoda*, *marina*, *spinifera*) it seems, I venture to think, to differ only, or at least mainly, in being superficially drawn out into those superficial, linear, capillary, and acute pointed processes (spines, Hertwig and Lesser).

In this form the authors did not prove the differentiation of the endo- and ectosarc, but of this there can be no doubt; it is nucleated (fig. 14, *n*), as need not be urged; the ectosarc showed several (as many as four in one individual, and on the side turned towards observation) contractile vacuoles (fig. 14, *c*, *c*); the pseudopodia extremely long, as much as five times the body diameter, and very finely granular.

Sphærastrum conglobatum, Greeff.[1]

seems to be identical with the form I myself previously described as *Heterophrys Fockii*.[2] As the author's account of it is very short, I venture here to give it in full. "Colonies of Actinophryan Rhizopods, mutually united by sarcode-strings. The individuals have a globular, sharply bounded body, from which the pseudopodia radiate, mostly not all round, but from such parts of the superficies as are directed outwards. Around the pseudopodia occurs a broad clear sarcode-envelope, as a rule showing a sinus reaching from one pseudopodium to another, and hence surrounding the whole colony like a garland. This outer sub[illegible] sometimes becomes gathered together about the [illegible] the apices of one or more of the larger [illegible] wing then a peculiar complication of [illegible] lines. The

[1] Greeff, loc. cit., p.
[2] Archer, 'Quart.

body of *Sph. conglobatum* consists of a clear homogeneous substance with numerous more or less coarse and fine granules. In the centre lies a comparatively large and clear globular body, with a darker nucleus, more readily coming to view by increased pressure. This form is mostly united into colonies of 10—20 individuals, each of which measures about 0·03 mm."

In the foregoing there is no mention made of "skeleton" nor "spicules," but the outer envelope presented itself to the author as "ein breiter heller Sarkodesaum"—so it appeared to myself, though doubtless wholly distinct from the body-substance in character and texture. Doubtless the "Gewirr von vielfach verschlungenen Linien" to be seen therein is just what I originally described as that sort of texture which presented a "streaky" appearance. No doubt Greeff's "grosse helle Kugel, mit dunklerem Kern," which I at first overlooked, but which is truly there, is to be regarded simply as nucleus and nucleolus. In my own experience this is not a very uncommon form, but by far more frequently occurs singly than combined into colonies, whilst such composed of 2—3—4 individuals occur more frequently than those of 10—20 (as described by Greeff), but even so large colonies as the latter no doubt sometimes present themselves. Touching Greeff's remarks as to the unequal distribution of the pseudopodia, it holds good to the fullest extent only in such as are so grouped together into colonies—single isolated individuals seem to give off the pseudopodia uniformly all round. Can a former figure of Greeff's[1] refer to this form occurring singly, at the time conjecturally thought to be a developmental state of *Acanthocystis viridis ?*

Whether this form has been studied by Hertwig and Lesser does not appear. Granting Greeff's view and my own as to the outer region being a mobile soft stratum (probably in young individuals only by degrees becoming, as it were, excreted and deposited externally), and devoid of hard parts (spicules), then surely it forms a strong case in point for the necessity of a group between the *naked* Askeleta and the undoubted Skeletophora. Here at any rate we have not the peripheral linear processes, forming in marginal view that fimbriated border to be seen in *Heterophrys myriopoda, H. spinifera*, &c., and which are claimed by Hertwig and Lesser as "spines," the genus Heterophrys being hence by them relegated to the Skeletophora. We have certainly the outer border running into subtriangular projections, the intervals between which produce those sinuses which cause

[1] 'Schultze's Archiv,' Bd. v, t. xxvii, f. 35.

Greeff to describe the outer border as "eingebuchtet." At the time I described this form I hardly thought it was essential to distinguish it generically from that with the fimbriated border, but possibly it would be as well that they should stand asunder in a generic point of view, yet side by side. If so, Greeff's generic name is very appropriate; but should the species not stand, according to the understood laws of nomenclature, as *Sphærastrum Fockii* (Archer), Greeff? My impression at the time and still is that it is highly probable that this Rhizopod may be one and the same with that figured but not named or described by Dr. Focke,[1] and referred to by him in his paper as "No. 1," and hence I called it after the name of that observer.

I have found this form in an encysted state, the inner body-mass becoming covered by a thickened wall, the outer region excluded therefrom, and the latter now looking like a delicate, much contorted and *crumpled* hyaline membrane, and destitute of granules; but this appearance is seemingly due to the manifold peculiar lines, already alluded to, which permeate it, and which are so difficult to be properly understood or interpreted. For a good representation of the appearance of this cast-off outer envelope see Greeff's figures in 'Schultze's Archiv,' Bd. xi, t. ii, ff. 25, 26.

Chondropus viridis, Greeff.[2] (Pl. XXII, fig. 20.)

Whether the very distinct-looking form named as above by Greeff should really fall in here, or along with the truly naked Heliozoan forms is not as yet clear. It is undetermined, I think, whether the marginal region shown in his figure (Pl. xxii, fig. 20) as of a yellow colour may represent an outer envelope comparable to the colourless and hyaline one of Astrodisculus, or the granular one of Heterophrys, or whether the whole basic substance of the body may have this tint.

The author himself only shortly describes it as "possessing a globular body filled with green, solid capsules, which upon compression under a high amplification presents an irregular considerably-fissured surface. Between the green capsules, mostly, as it appears, at the outer region of the body, [illegible] lie minute, sharply contoured, bacillar bodies (Stäbchen) and other more or less irregular particles [illegible] contour. The sarcode surrounding th[illegible]

[1] Siebold and Kölliker's 'Zeitschrift für wi[illegible] 1868.

[2] Greeff, "Ueber Radiolarien und Radi[illegible] Süssen Wassers," in 'Schultze's Archiv,' [illegible]

yellow appearance, which, especially at the outer circumference, appears as a yellow border. The sarcode likewise carries many dark, shining granules, surrounding the surface, and in vigorous movement. From the outer circumference of the body there project in a radiant direction delicate filamentary pseudopodia, scarcely so long as the diameter of the body, in which the refractive and comparatively large granules emanating from the surface, and from the interior run up and down with surprisingly great rapidity. A nucleus, or central capsule-like structure (says the author) I could not perceive."

The fact that the author's figure shows the pseudopodia as passing down, and continued directly through the yellow border can, I fancy, be interpreted in two ways: *either* that the yellow border represents an outer investing envelope (compare the figure in this regard with Astrodisculus, Greeff, Pl. XXI, fig. 3, or the author's own Astrococcus, fig. 2, or with *H. variabilis*, Pl. XXII, fig. 15)—*or* the continuation inwards of the pseudopodia (fig. 20) may represent a prolonged axile thread comparable to that of Actino-sphærium, or better that of *Raphidiophrys pallida*.

I suppose that the latter would be rather the author's opinion, so far as we can judge from his brief account of it. Were this the true explanation the form would seem to fall close along with Actinophrys, for it is most probable that only the opaque contents prevented the author from perceiving a nucleus. The author does not enter into any more precise description as to the nature of the green "capsules" (query, chlorophyll-granules? but the tint of the figure is not that of chlorophyll), nor of the bacillar bodies ("Stäbchen"). The author found this pretty form in spring, in sandy river mud, filled with diatoms. It is to be hoped he may refind it in the same locality, and be able to submit it to fresh examination and experiment.

Heliozoa Skeletophora.

In this group a greater number and variety of forms present themselves than in the *Askeleta* or in those which I here suggestively denominated *Chlamydophora*, the distinguishing generic and specific features here being in the main due to the differences in the formation of the "skeleton," whilst the specialities of the soft parts (the body-mass and the pseudopodia), as well as, to some extent, the colour, offer holdpoints (though no doubt not so readily grasped) to assist in their systematic arrangement.

Except that the endosarc it is which contains the nucleus

—the ectosarc, the contractile vacuoles—there is not much in common with the corresponding portion of the body-mass in the Askeleta.

Unlike Actinosphærium, where, as is seen, the reverse state of affairs prevails, Hertwig and Lesser point out that in the Skeletophora (including also the forms I have proposed to group as Chlamydophora) the ectosarc, which is permeated by coarser or finer granules, is the portion of the substance into which the nutriment is taken, and in which assimilation is effected, the nutritive particles never reaching the central part. This latter appears to be, therefore, of a finely granular or homogeneous nature, of pale greyish-blue tint, a more or less marked line of demarcation often evident, but this *not* due to any special membrane mutually limiting off the two regions.

But that in all the skeleton-bearing Heliozoa the structure is to be interpreted as Hertwig and Lesser thus lay down is, I fancy, not to be regarded as decided. To my eyes, as yet, the body-mass from the nucleus outwards to the bases of the pseudopodia does not always show any very sharply marked differentiation of consistence (though, on the other hand, *Acanthocystis spinifera*, for instance, does so); whilst the fact that the incepted food does not occupy the centre of the body-mass may be due to the hindrance afforded by the subcentral, rigid, sometimes comparatively large, nucleus. There are some species, indeed, in the substance of which crude food of any kind (diatoms or the like) is rarely, and in others I fancy one might almost say is never, to be noticed.

As regards the pseudopodia, the Heliozoa Skeletophora, just like the Monothalamia, present various species in which, as may be seen, these characteristically appear granular or homogeneous, branched or unbranched; anastomosis of neighbouring pseudopodia sometimes, but not often occurs. The pseudopodia of some forms, according to Grenacher[1] and Greeff,[2] present a slender axis (like Actinosphærium) of the greatest delicacy.

In the different species the granular or non-granular nature, as well as the comparative thickness (one might better say the *thinness*) of the pseudopodia seems to me to be a very constant characteristic, whilst quite as much so is also the average or the extreme relative length of which they are capable in the various forms. Of course this remark applies to examples in a normal and undisturbed state

[1] "Bemerkungen über *Acanthocystis viridis*" in 'Zeitschr. für wiss. Zool.,' Bd. xix, p. 292.

[2] 'Archiv. für Mikrosk. Anat.,' Bd. v, p. 484.

for some time under observation, and after having recovered the shock of removal from the gathering to the slide, and of putting on the covering glass.

When the pseudopodia carry granules, these are generally of greater diameter than the pseudopodia themselves, and hence cause little swellings; when these occur, several in succession, a submoniliform or somewhat torulose aspect is (temporarily) imparted to the pseudopodium; sometimes in some forms little fusiform thickenings of the pseudopodium itself may occur, which may slowly travel up and down.

Hertwig and Lesser divide the Skeletophora into two subgroups, their distinctions based on whether the skeleton consists of numerous loose pieces, this subgroup they designate Chalarothoraca—or of a solid and connected shell, which subgroup they designate Desmothoraca. In the

Chalarothoraca, Hertwig and Lesser,

the individual pieces forming the skeleton are in the different species of various forms. They may be, as has been said, either elongate and pointed and scattered, when they are called "spicules;" or radial, when they are called "spines;" or they may form little spherules or tablets, or some of these varieties of skeleton-constituents may be characteristically associated in one and the same species. During inception of food these parts can become pushed aside so as to leave it free opportunity to become passed into the soft body-mass; so also do they seem to become *shunted* during "conjugation" or mutual temporary junction of the sarcode of two individuals, as can be seen in Acanthocystis. Excrementitious matter, on being expelled, acts in the same way in pushing aside the loosely associated spines, which soon thereafter regain their position.

Acanthocystis turfacea, Carter.

Whilst as mentioned I can quite coincide that the genus Acanthocystis, subordinated to the Radiolaria by its discoverer and other subsequent observers, should not be so but is simply a skeleton-bearing Heliozoan, I cannot at all feel satisfied that Carter's original *Acanthocystis turfacea* can be specifically identical with either the so-called *Actinophrys viridis*, Ehrenb., or *A. brevicirrhis* (Perty), the short fimbriate border of either spines (?) or pseudopodia (?) around the periphery of Ehrenberg's figure of the former is so completely unlike Carter's not very uncommon form, hence I must hold that Greeff and Grenacher are wrong in identifying the form therewith and calling it *Acanthocystis viridis;* whilst as regards *A. brevi-*

cirrhis, Perty, I should strongly suspect its identity with my own *Acanthocystis Pertyana*. Could this latter, indeed, be proven (but it probably never can) the form should be of course designated *Acanthocystis brevicirrhis* (Perty), Archer. Greeff's *A. pallida* must, I suppose, be merely an accidental colourless form of *A. turfacea*.

Hertwig and Lesser point out that the basal-plates of the spines do not lie in absolute contact with the superficies of the globular inner sarcode body, whence originate the pseudopodia; that is to say, the bases of the spines are held apart from the inner sarcode-body, slightly above the level of the origin of the pseudopodia; the latter are thus carried a very short distance upwards, through a very narrow zone seemingly *empty* (except of water?) everywhere surrounding the body and intervening between its surface and the interior of the globular space formed by the aggregate "skeleton," before passing outward (through its minute intervals) into the surrounding medium. This being so, those authors aver they cannot coincide with me in accepting any protoplasmic stratum external to the inner body-mass (giving off the pseudopodia and in some forms enclosing the chlorophyll-granules). They say there may be certain protoplasmic emanations given off from the pseudopodia connecting the spines together. But can any one examine our *Acanthocystis turfacea* from time to time and not perceive the cloudy, granular (possibly indeed somewhat incoherent-looking), certainly, as compared to the body-mass, much softer, substance in which the spines are more or less immersed? Nay, is it not depicted in several of the figures of Acanthocystis extant?[1] Nay, how could this "skeleton" possibly "come there" if it be really altogether external to the *living part* of the organism? I should rather suppose that the seeming vacuous interspace around the body may be also occupied thereby, but here of an extremely hyaline nature (so also Pompholyxophrys and others). Indeed I should be inclined to hold that this outer envelope has its parallel in other forms, (*Heterophrys*, *Raphidiophrys*). But it may be indeed that it is in some forms to a certain extent or perhaps sometimes wholly evanescent, and that a certain epoch it may have (dissolved? and) disappeared. In the form which (perhaps erroneously) I referred to the genus Heterophrys [illegible] envelope is shut out when the inner [illegible] into the encysted state.

The authors were unable to s[illegible] cation between endo- and ecto[illegible]

[1] 'Archiv für Mikrosk. Anat.,' [illegible]

but they noticed the homogeneous character of the median region as distinguished from the granular outer part bearing the green or colourless corpuscles; they noticed also the contractile (but non-protuberant) vacuoles in the outer region, although Greeff has not seen their contraction. I had long overlooked the nucleus,—carmine fluid readily indeed discloses it. Hertwig and Lesser more correctly describe it. Grenacher overlooked it, though Greeff disclosed it by pressure, but without arriving at a sense of its nuclear nature. The latter author speaks of certain "manifestly cellular structures"[1] which, so far as I can see, Hertwig and Lesser very correctly deny, and refer what met Greeff's observation to simple vacuoles. Moribund or half effete sarcode of many forms is in fact prone to form "vacuoles" surrounding some of the granular structures or corpuscles, simulating the appearance of "cells" with "nuclei."

Grenacher records the observation of "a rather large cavity of irregular contour, apparently filled with a watery fluid, which assumed a stellate figure whilst producing sinus-like inlets of the plasma." . . . . "Precisely in the centre of this cavity there occurred without exception a little pale corpuscle, whence numerous, likewise pale threads run out like rays in all directions."[2] These fine radiating lines Grenacher regards as prolongations of the (presumed) axile threads of the pseudopodia. Greeff believes he has found the same structure.[3] He speaks of "a clear round or oval vesicle almost in the centre of the Acanthocystis from which the remarkable stellate radiating-system of fine threads proceed."

This peculiar central structure in *A. turfacea* Hertwig and Lesser have been unable to detect; that so able observers have not succeeded in refinding it I almost feel glad, for I as yet have not been able to see it. But the opaque character of this form, so densely loaded as it is with green and dark granules, is well calculated to prevent an observation in this regard. The nucleus, however, is not difficult of detection, once one *knows it is there*. See, however, Schulze's description hereafter of quite a similar internal structure in his *Raphidiophrys pallida*.

Hertwig and Lesser record:—Certain examples of this species, otherwise normally developed, presented themselves more or less closely covered externally with colourless, very refractive, globular bodies, about equal in size to the chlorophyll-granules. These shining bodies were laid closely about

[1] Loc. cit., pp. 290, 292, 293.
[2] Loc. cit., p. 490.
[3] Loc. cit., p. 487.

the basal plates of the spines, but without being attached to them. They frequently moved in a tremulous manner, in little oscillations, or passed up and down by the pseudopodia; the authors even saw some of them, wholly detached from their place, betake themselves, with a vigorous movement amongst the spines, to some other part of the superficies. They could perceive nothing of cilia or other motory organs. There was no perceptible attachment of the globules to the body; they once, however, saw some of these, along with two of the spines, pass upwards by a pseudopodium, but in connection with neither, yet with the same rapidity as the spines. It gave them the impression as if these were drawn forwards by an extraordinarily delicate, imperceptible, thread of protoplasm. The authors were quite unable to offer any explanation of this phenomenon, merely recording the observation in the mean time on account of its curiosity.

Could this be a parallel (and equally puzzling and inexplicable) for the observation I myself recorded[1] as regards an as yet unnamed rhizopod (scarcely Heliozoan), where certain corpuscles were suddenly and simultaneously "shot off" in a volley all round the periphery of the body-mass, then somewhat slowly retracted and *reimbedded* therein? Unless these corpuscles were retained and drawn back by (invisible) protoplasm-threads, they would, one would suppose, have been projected beyond recall.

A. spinifera, Greeff.[2] (Fig. 8.)

In this species (whose structure and details are now so well known, it is unnecessary here to enlarge thereupon) it was that Hertwig and Lesser were able most clearly to see the evident line of demarcation between endosarc and ectosarc. Into a mistake in this regard doubtless I myself have fallen in taking this inner region for "central capsule;" and I certainly now feel I have done so in taking the true *nucleus* for the representative of the "inner vesicle" (*vesicula intima* or "Binnenblase"). Nor am I alone in this, for Greeff shortly afterwards, I find, had propounded the same views. But Greeff went further and stated that the pseudopodia were traced by him to be continued inwards, and to be in connection with this inner region, his "apparent equivalent to the central capsule," being one and the same with Hertwig and Lesser's endosarc. Could what Greeff records he was able to perceive thus so far reaching inwards have been an "axis" equivalent to that of Actinosphærium? if so, that

[1] 'Quart. Journ. Micros. Sci.,' n. s., vol. xv, p. 114.

[2] Greeff, in 'Schultze's Archiv,' Bd. v, p. 493, t. xxvii, f. 20, 21.

must have been indeed of almost inconceivable fineness, for in this form the pseudopodia themselves are of extraordinary tenuity. Greeff does not appear to have detected the true nucleus = my then supposed "vesicula intima," but, as will be seen, Hertwig and Lesser have detected therein a nucleolus.

Those authors constantly found in this species, commingled with the usual fine granules, chlorophyll-granules (or, as they say, "oval chlorophyll-green granules"), never the yellow globules seen by Greeff and myself. I have since myself found this form with only just a few chlorophyll-granules—never the abundant quantity mostly characteristic of *A. turfacea;* whilst, on the other hand, the yellow globules, which are most probably of oily nature, certainly of that appearance, are not always present. As Hertwig and Lesser remark, very likely correctly, this fact is most probably to be accounted for by the varying degree of nutrition of the organism. They also describe the not frequently-to-be-observed external contractile vacuoles, which do not, however, cause any projection of the periphery. They have found the nucleus to be somewhat excentric, of a broadly elliptic figure, and, by the application of weak acetic acid, to contain a nucleolus; under this treatment the latter (not the nucleus itself) assumed a granular appearance (fig. 8, *n*), the endosarc (fig. 8, *k*) also assumed a similar but more coarsely granular appearance, the ectosarc (fig. 8, *r*) comparatively unaltered. On applying concentrated acetic acid the latter swelled up, accompanied by a gradual solution of the contained colourless granules (perhaps also of the coloured), a change communicated by degrees to the nucleolus, both it and the nucleus now appearing throughout as a homogeneous pellucid vesicle (see fig. 8). In those extremely minute representatives of this form sometimes to be encountered, Hertwig and Lesser have not been able to demonstrate these structures.

Acanthocystis aculeata, Hertwig et Lesser.[1] (Fig. 6.)

Provided, as characteristic in the genus, with a basal-plate, the spines, like those of *A. spinifera*, are here subulate and acutely pointed, but they are stouter than in that species, and are more or less curved. The spines are somewhat irregularly posed and pointing various ways, their regular radial arrangement being seemingly disturbed by the interposition of minute bacillar pieces lying tangentially, slightly curved, corresponding to the globular periphery of the body. As to the body-structure—nucleus, endosarc (fig. 6, *k*), ectosarc (*r*), which

[1] Loc. cit., p. 201, t. iv, f. 2.

shows some non-prominent contractile vacuoles, pseudopodia —what applies to *A. spinifera* holds good here. The authors regard the colourless or coloured rounded corpuscles met with in the ectosarc as due to the gradual breaking up of the food during the digestive process, and they record indeed having traced the transformation of such into these corpuscles; at first during the breaking up of the incepted bodies they supposed they had encountered a more or less advanced process of self-fission, but they afterwards found this was an error. They regard indeed the chlorophyll-granules, when present, just like these colourless corpuscles, as due to the inception and breaking up of algal organisms, and go so far as to suggest that in all cases the green granules in Heliozoa owe their origin to algæ incepted from without. In this view I would myself as yet not at all feel inclined to coincide; in such forms as *A. turfacea*, *Raphidiophrys viridis*, the intra-peripheral layer of chlorophyll-granules appears so comparatively constantly and seemingly characteristically; even when these forms are found colourless these same granules seem still to be there, if I mistake not, but deprived of colour—if I might venture to suggest, somewhat starchy in appearance. It is, no doubt, possible (perhaps not unfrequent) that such rhizopods may incept numerous green algæ and their zoospores, &c.; such examples would offer a green appearance until *digestion* had set in, but this would be merely exceptional and altogether different from the aspect of the *habitually green* forms.

This seems a perfectly distinct species; it has not, however, met my own observation.

Acanthocystis flava, Greeff. (Fig. 7.)

To this genus Greeff adds another seemingly well-marked species under the above name.[1] It is characterised by its yellowish-brown colour, showing in the interior some red or dark brown granules, as well as some of larger size colourless. It possesses a median globular, apparently hyaline central body, doubtless nucleus, designated "central capsule" by the author. The radial spines are scarcely a third of the body-diameter, comparatively broad at the basis, gradually attenuated upwards and acute at apex, and are seated on the surface of the body by the usual basal plates. The pseudopodia appear to be about one and a half times the length of the body-diameter.

[1] Greeff, loc. cit., p. 17, t. i, f. 5.

Pinacocystis rubicunda, Hertwig et Lesser.[1] (Fig. 10.)

Although the object of the present *résumé* is to bring before the readers of this Journal the recently discovered rhizopodous forms of the freshwater only, that which Hertwig and Lesser describe under this name, found in sea-water from Cologne, comes so close to those characteristic of the former element, it could not properly be omitted. And this the more especially as the authors call to mind that the representatives of the Heliozoa known to appertain to the sea are but few, hence tending to indicate the erroneousness of the ordinary appellation "Freshwater Radiolaria," and to show how little it accords with the geographical distribution of the Heliozoa to regard them as a group in the course of "natural selection" as it were halting behind the marine forms. In this point of view *Heterophrys marina* occurring in fresh water, as mentioned, is also interesting.

This form as regards the "skeleton" might be compared to an Acanthocystis, *without* "spines," however, *but with* the "basal-plates" appertaining to that genus, the skeleton being formed of globularly arranged round tablets, without appendages. The structure of the body-mass agrees with that of that genus—the absence of demonstration of contractile vacuoles the authors suppose might have been due to unfavorable examples; the peculiar sienna-brown colour of the granules would only at best be of specific value. The pseudopodia are less numerous and shorter than in the species of Acanthocystis, but they otherwise resemble. In fact, the authors felt inclined to embrace this form in that genus (slightly expanding, of course, its limits), and refrained mainly on account of the fact that the name necessarily conveys that the skeleton is composed of *spines*.

Pinaciophora fluviatilis, Greeff.[2] (Fig. 5.)

(found as yet by the author only in freshwater streams), in a generic point of view comes very close to the foregoing, though, no doubt, it is quite a distinct species. It is characterised like the preceding by the possession of peripheral plates, closely arranged in a hollow-globular manner; but here these are not round and flat, but of a compressed oval figure with pointed ends, thus broader in superficial view than when seen towards the "equator" of the rhizopod; they are, moreover, according to the author, perforated by numerous fine foramina, appearing as fine lines running

[1] Loc. cit., p. 209, t. iv, f. 5.
[2] 'Schultze's Archiv,' Bd. xi, p. 26, t. i, ff. 15, 16, 17.

in a radial direction as regards the inner body, and through which he supposes the pseudopodia, which seem very fine and delicate, to project. If the dotted appearance presented by the plates be really due to the presence of actual foramina, probably this should remain generically distinct from the foregoing. They may possibly not be perforate, but merely indications of differences of density in the plates, and the pseudopodia pass between, not through them; at least the supposed foramina are greatly more numerous than the pseudopodia. But they certainly have the aspect of perforations. In any case the form of the plates is very distinct from those of Hertwig and Lesser's species. There is, no doubt, strong enough resemblance to justify the idea that this and the foregoing are congeneric; and in case of such view being adopted the name Pinaciophora has the precedence, as Greeff would appear to have already briefly described his form in 1871,[1] considerably prior to the publication of Hertwig and Lesser's memoir.

The plates, like those of Hertwig and Lesser's marine form, are not firmly connected together, although when *in situ* are in contact and overlapped, but they can readily be separated and isolated by pressure. Immediately below the hollow-globular skeleton formed by them, the author describes "a narrow clear sarcode-layer surrounding the reddish-brown coloured body-mass. Ecto- and endosarc are not sharply distinguishable. In the middle there lies a comparatively large hyaline capsule-like spherical body, which again contains a second likewise globular and centrally posed structure, enclosing finely granular contents." The latter is doubtless the nucleus, with nucleolus.

Raphidiophrys, Archer.[2]

In this genus, as in the preceding, Hertwig and Lesser are equally disinclined to admit that the rhizopod possesses external to the central body-mass any special sarcodic stratum. They call this region here, as in Heterophrys, simply "skeleton." Without doubt we have here to do with a true (chalarothoracous) "skeleton," and this consists of elongate, nearly straight, or gently curved and acutely pointed, or (in the author's new species, *R. elegans*) considerably curved and somewhat bluntly pointed spicules, but to me such "skeleton" parts seem to be immersed in an outer mobile matrix of *some kind*. That, to a great extent,

[1] "Sitzungsberichte der Gesellschaft zur Beförderung der gesammten Naturwissenschaften zu Marburg," June, 1871.

[2] 'Quart. Journ. Microsc. Sci.,' n. s., vol. ix, p. 255.

it is the same in kind as that of Heterophrys I fancy must be true; it is more mobile, more homogeneous-looking—that is to say, less striate and granular-looking—than in that genus; but the one great distinction seems to be its possessing spicules which are absent in Heterophrys (leaving out of view the peripheral capillary processes claimed as "spines" by Hertwig and Lesser). And with what I venture to hold some amount of contradiction, after having defined the heliozoan as composed of but one stratum only, surrounded by a skeleton formed of numerous spicules, these authors proceed to speak of the sparing protoplasm-medium cementing and holding together the pieces of the skeleton. In describing *R. elegans* they say that "between the spicules they were able to perceive, especially after adding acetic acid, sparing granules, and they regard these as slight traces of protoplasm originating from the pseudopodia, and combining the loosely disposed skeleton pieces." This seems, I venture to hold, to be yielding the question in dispute, and I should suppose the admissions capable of being extended to other genera. If this, after all, undeniably soft-connecting medium owes its origin to the pseudopodia, they at all events pass up from the central inner body-mass through and through this outer spicule-carrying region, appearing during the whole distance *quite sharply marked* and *without any evidence* of becoming gradually softened or dissolved, or in fact at all modified in nature. In either *R. viridis* or *R. elegans* when an example is first placed on a slide, one at first sees the outer spicule-bearing region quite flaccid as it were, and the contained spicules "tossed" irregularly, but with a great tendency to lie very much in a tangential direction; in a short time, when the organism begins, as one might suppose, to recover the shock of the transfer from the "collecting-bottle" to the slide, this outer region (in *R. viridis*, to my eye, as well, offering a more or less evident degree of very light purplish-brown tint) begins to stand off in a number of radial somewhat conical prominences, and the spicules become more or less tilted on end, and masses of them become piled in conical heaps, and clustered about and as it were, more or less, lying obliquely against certain of the pseudopodia, whilst in *R. elegans* they assume more and more of the curious *festoon-like* arrangement seen in that species. All this *they* could not do spontaneously, one must suppose, but rather they obey the innate *movement* of the *medium* in which they are immersed. Doubtless the elongation of the pseudopodia must and does contribute to effect this, by assisting to carry upwards the piles of spicules

through which they directly pass. If, again, there were no living medium (sarcode or some modification of it), surely the spicules would readily get removed and lost, but they stick together with wonderful unanimity.

Further, surely it would not be supposed that these numerous spicules could originate *actually apart* from the *living* portion; it will not be held that they came into existence independently, and that the pseudopodia merely lend a little of their own substance just to prevent the multitudes of loose spicules already circumjacent becoming lost. Is it not more natural, rather, to assume that this outer cloudy-looking region must have really preceded the existence of the spicules, and that possibly it in turn may owe its origin to an exudation from the whole body-mass (like the gelatinous investment of algæ); but be this as it may, until we shall know the whole development of such a form, our generic characters can be based only on the (mature) *fully formed organism as we find it*. And after all I still venture to suppose my original conception of the conditions under consideration not to be very far from the truth.

So, to a certain extent, Hertwig and Lesser, I take it, misconceive on one point what I meant to convey. I quite acquiesce that the seat of individuality is to be sought for in the sarcode body-masses, and that no matter how many in one group such an assemblage is to be regarded not as an individual but as a *colony* of individuals; and it is probable that one or more of such could become, as is probably often the case, dissociated by a breaking off of the sarcode-connecting isthmuses, and at the same time bringing away a share of the *common* outer envelope with the contained spicules, and then maintain an existence apart.

Raphidiophrys pallida, Schulze.

Under this name Schulze describes a form (found in the basin of the Botanic Garden at Graz, on half-decaying Ceratophyllum leaves),[1] primarily distinguished from *R. viridis*, Archer, by the want of chlorophyll-granules, and hence its colourless character. But, seemingly, just this circumstance enabled the author to gain an instructive insight into its inner structure, which he describes.

Whilst *R. viridis* usually occurs in colonies, this species constantly presented itself singly. The globular body-mass is surrounded by an either directly apposed or slightly remote stratum of spicules, tossed about in all directions

[1] Schulze, in 'Schultze's Archiv,' Bd. x, p. 377, t. xxvi, fig. 1.

from tangential to approximately radial, and often forming conical heaps projecting outwardly. From the generally smooth surface of the body proceed radially slender filiform pseudopodia passing through the skeleton-layer; they neither branch nor anastomose; they are straight, finely pointed, and mostly in length considerably exceeding the diameter of the body.

Amongst the numerous minute granules imparting a cloudy appearance to the body-substance, some larger, pale, shining corpuscles can mostly be distinguished irregularly distributed towards the outer part of the body, but not at its extreme limits. The author describes them as corresponding in figure, size, and position to the "so-called" chlorophyll-granules of such Heliozoa as *Raph. viridis* and *Acanth. viridis*. He supposed that these increased in quantity with increased nutriment, and that they disappeared with long fasting.

He demonstrated the presence of a distinct nucleus in the interior of the body. It strikes the observer, on account of its large, strongly refractive, homogeneous and hence somewhat shiny nucleolus (of about 0·01 mm. in diameter), which appears to be either smooth and roundish or oval, or tuberculate, or even indented. The nucleolus appears surrounded by a narrow border, marked off so sharply against the surrounding plasma as to give the idea of possessing an enclosing membrane (Kernmembran). The nucleus as a whole is oval or egg-shaped, more rarely globular, and has a diameter of about 0·015 mm. It lies very excentric, indeed close to the periphery, mostly with its longer axis in a radial direction. Extremely rarely the author saw two nuclei.

On seeking for a cause of the very excentric position of the nucleus, the author was surprised to discover that here, as well as in *Acanth. viridis*, according to Grenacher and Greeff's descriptions, "a skeleton is present, consisting of fine spines standing radially and uniting in the centre in a stellate manner."

In *Acanth. viridis* (I believe identical with *A. turfacea*, Carter) Grenacher described in the centre of the body "a somewhat large, irregularly stellate cavity, apparently filled with watery fluid." In the middle of this he found "a little pale, roundish corpuscle, from which ran in all directions radially numerous likewise pale, straight threads." Athough he could not follow them, Grenacher supposed these were in connection with the delicate axile threads of the pseudopodia. A similar connection of "axile threads" with a similar central structure Greeff thought he saw in *Actinophrys sol.*

Schulze's experience of the structure in *Raphidiophrys pallida* accords with the descriptions of those authors.

Precisely in the centre of the body he found a minute round body (0·003 mm.), from which passed very fine straight "spines" in all directions radially through the substance, and he could follow them into the pseudopodia. He was able to see this particularly plainly in an example killed by sulphuric acid and become swollen and hyaline, although the pseudopodia were drawn in and thus greatly degenerated. He could see nothing central space described by Grenacher in *Acanth.* "radial spines and the minute central globu they all unite could be well recognised." called) "spines" and the globular conne siliceous, any more than the (so-called by Schu "spines" of the pseudopodia in Actinosphæriu satisfied himself by application of reagents.

A further characteristic of the body-substance in *A* is the presence of numerous (10—20) pulsating space with clear fluid. These are not, as usually the case, r but are rather, as a rule, longish egg-shaped, and so pl that the longitudinal axis stands radially, the narrower directed inwards. They appear, as it were, just as if caugh between the radial "spines." They stand near, sometime very close to, the outer part. He could see no membranous wall to these "pulsating vacuoles."

Such matters as food, diatoms, minute algæ, &c., were often present, lying in the outer region, being naturally hindered from passing into the middle by the concurrent system of radial "spines."

The siliceous spicules are solid, cylindrical, slightly curved at both ends, gradually and equally attenuate and pointed. They are thus similar to those of *R. viridis*.

After stating that he could not satisfy himself of the presence of any "shiny, fluid, sarcode, cortical layer," he nevertheless goes on to say, "Certainly the spicules of *R. pallida* are here and there loosely united by means of a soft sarcode, as well with the pseudopodia making their exit between them as with one another, but I can perceive in it no continuous outward cortical layer, but only the softer sarcode covering of the 'pseudopodial spines' which flows over from the globular inner body on the latter, and from there on the siliceous spicules standing in contact with them, uniting them as well with the pseudopodia as also amongst one another, loose and easily movable. . . . . I assume that a number of vacuities may be left between the siliceous

spicules, through which the water can freely pass to the surface of the globular body." And he points to the seeming vacant interval between the spicular stratum and the surface of the body as an evidence that there is no compact sarcode-mass surrounding the body.

Having already defended my own views I need not even briefly recapitulate; but really, although Professors Hertwig and Schulze seem apparently opposed to my view, I venture to think it is only *apparently*, and that the views, at any rate, of the latter, as expressed above, admit all I had contended for. The peripheral portion of the body-substance is the same in nature and consistence as that which forms the pseudopodial envelope, and could, I fancy, equally take a share in giving off the possibly lacunar, certainly very delicate and soft, stratum, forming, as it were, a matrix for the spicules. The space immediately exterior to the globular body is usually without spicules, but I query if it is absolutely and everywhere destitute of Schulze's admitted "weiche Sarcode."

The spicules must have taken origin within living matter, and the whole stratum may certainly by degrees in greater age get pushed aloft, by-and-by leaving a void region below it.

As regards points of difference between this form and *R. viridis*, they strike me as specifically important, apart from the absence in the former of the chlorophyll-granules. It is true that in *R. viridis* these are sometimes colourless and, as it were, gradually becoming starchy in appearance, but they are mostly present in great abundance and prevent any insight into the interior. In *R. pallida* the pseudopodia are seemingly granuliferous, and show here and there fusiform thickenings. In *R. viridis* they are not granular, but homogeneous. In fact, with *black ink* it is really not possible to give on paper a correct representation of them; an extremely fine line of weak gum-water would be more true. Again, they are a great deal longer than Schulze depicts for his *R. pallida*, and I think amongst the most important and special of the idiosyncrasies evinced by particular rhizopods are those inherent to and shown by the pseudopodia. Again, *R. viridis* never has shown pulsating vacuoles. Further, I have never noticed its containing *any* incepted food. Its opacity could act as a hindrance to the perception of such an inner system of radiating "spines" as Schulze describes for *R. pallida*, but I would by no means say it does not exist. A nucleus cannot be made out in the body-mass of *R. viridis* in the ordinary state, but the application of carmine solution

discloses sometimes more than one in each *individual*. The two species agree as mentioned in the form of the spicules, but the outer region in *R. pallida* appears colourless, in *R. viridis* it appears of a very pale purplish tinge. Taken, then, altogether, I think Schulze is quite justified in regarding his form as a distinct species.

Raphidiophrys elegans, Hertwig et Lesser. (Fig. 19.)

This very interesting species, which I myself have taken but on one single occasion, is, indeed, very unlike *R. viridis*, though doubtless must be relegated to the same genus. It is much more likely to be taken, at first glance, for a colony or group of the form I referred to Heterophrys under the name of *H. Fockii*,[1] which latter, I venture to suppose, is identical with *Sphærastrum conglobatum*, Greeff,[2] of which mention has been made above. But the presence of the peculiarly curved spicules will alone distinguish them. These are much more curved, shorter, more bluntly pointed than in *R. viridis*. When the specimen is some time on a slide, one sees the conical lobe-like extensions of the outer region become more prominently projected, and the little more than semicircularly curved spicules take a very pretty continuous festoon-like arrangement, as it were, mutually interlinked, and stretching from one pseudopodium to another.

Hertwig and Lesser describe the globular body-mass (which I may say is considerably smaller than in *R. viridis*) as mostly, though not always, richly laden with chlorophyll-granules; in their figure only a very few are shown. In my examples there were none present. Almost in the middle of the globular body-mass there is a nucleus (with nucleolus); the authors say this occurred to them sometimes of a biscuit-shaped figure, but they did not see any subsequent subdivision of it. They saw no contractile vacuoles (just as little as they appear to occur in *R. viridis*), nor could the authors perceive any marked differentiation into endo- and ectosarc.

However, from the subperipheral arrangement of the chlorophyll-granules (notably in *R. viridis*), it is probable such a differentiation exists. In *R. elegans* the pseudopodia appear richly granuliferous—in this *quite unlike* those of *R. viridis*, in which they occur as delicate lines of great and equal tenuity throughout, perfectly homogeneous and colourless, "silvery" in appearance, and absolutely straight, and of very great length (seemingly several times longer than those of *R. elegans*).

[1] 'Quart. Journ. Micros. Sci.,' n. s., vol. ix. p. 267.
[2] 'Schultze's Archiv für Mikrosk. Anat.,' Bd. xi, p. 20, t. ii, f. 24.

As in *R. viridis*, though occasionally occurring singly, the individual body-masses of *R. elegans* occurred sometimes to the authors combined in colonies; on the only occasion I have met this species so they occurred to myself; as they describe, the body-masses are mutually united by slender isthmuses of sarcode, yet vastly thicker than pseudopodia, and the whole group immersed in and surrounded by the common spicule-bearing matrix. In such a group, of course, the pseudopodia are mainly given off from the outer portions of the component individuals, though some may be given off inwards, and then may cross, and even inosculate.

Pompholyxophrys punicea, Archer.

Hyalolampe fenestrata, Greeff.

Touching the priority of the nomenclature for the foregoing well-marked and not uncommon Heliozoan, I venture to think it must be conceded to my name, for although Greeff's and my papers were published in the same month, the Number of the 'Quarterly Journal of Microscopical Science' was published on the 1st, and if I mistake not the Heft of the 'Archiv für Mikroskopische Anatomie' did not make its appearance till the middle of the month, in which case I should have been in advance only by about a fortnight.

Hertwig and Lesser without doubt correctly regard the outer stratum of silicious spherules as a skeleton, and in opposition to Greeff, they equally correctly regard these as but loosely connected and readily dissociated (either permanently by pressure, or temporarily, or even a few permanently, during inception of food or extrusion of fæcal matter). If some pressure be exerted on an example of *P. punicea* (*H. fenestrata*, Greeff), so as to break up the body-mass into two portions, each soon appears again rotund and surrounded around the periphery by its *share* of the spherules (perhaps now only for the most part in a single stratum); still no doubt some are permanently dissociated by the force employed, yet that any at all almost should accompany the disconnected portion of the body-substance and become redistributed around it with so much nicety could, one would suppose, be effected only by being carried over along with and in *some* medium; it at least proves that Greeff's original conception was so far inaccurate, that is, that it does not possess a desmothoracous "fenestrate" skeleton.

Hertwig and Lesser consider the body-mass, densely loaded

with reddish granules, as equivalent throughout—that is, they could perceive no differentiation into endo- and ectosarc, but there appear to exist a few non-contractile vacuoles. Possibly the coarsely and very densely and opaquely granular substance of the body may render such a characteristic all but impossible to discern. Certain it is that there is a nucleus which I formerly overlooked, which the dense nature of the substance is well calculated to obscure, but it is readily revealed by carmine solution. Greeff was the first to recognise this body. Hertwig and Lesser rightly draw attention to the great delicacy of the pseudopodia, their granuleless nature and their seeming fewness; yet this form, amongst Heliozoa, is one of the most vigorous as regards powers of locomotion; one can often readily enough see it roll along with no very little celerity for *such* an organism.

The authors describe a biscuit-shaped constriction of the body-mass. In one case they saw two bodies united by a narrow isthmus of sarcode, *each* possessing its nucleus.

Greeff has described an encysted state of this species in which a porous siliceous coat is formed around the inner body. This must be similar to what I have myself observed in the case of *Sphærastrum conglobatum*, Greeff = *Heterophrys Fockii*, mihi (see above).[1]

H. exigua, Hertwig et Lesser,[2] as mentioned, seems to be mainly distinguished from *P. punicea*, Archer, by its greater minuteness (?), the greater delicacy of the "skeleton," the component spherules of which are more minute and superimposed in more numerous layers; it mostly contained one pre-eminently large, ruby-red pigment granule; a nucleus, with nucleolus, present; very delicate, homogeneous pseudopodia. As far as is requisite this form has already been referred to under Astrodisculus (*ante*). It might seemingly be looked upon as a merely diminutive form of *H. punicea* (?).

I postpone till next number the consideration of further lately described new rhizopodous forms.

[1] 'Marburger Sitzungsberichte,' June, 1871.
[2] Hertwig and Lesser, op. cit., p. 222, t. iv, p. 6.

On the Coincidence *of the* Blastopore *and* Anus *in Paludina vivipara.* By E. Ray Lankester, M.A., F.R.S. (With Plate XXV.)

Dr. N. Bobretzky, of Kiew, to whom we owe invaluable works on the developmental histories of Astacus, Palæmon, and Oniscus, has recently published in the 'Archiv fur Mikrosk. Anatomie' (vol. xiii, p. 95) a memoir entitled 'Studien über die embryonale Entwickelung der Gastropoden." His observations relate almost exclusively to the genera Nassa, Natica and Fusus and were made in Dr. Dohrn's Zoological Station at Naples. Six plates illustrate this work, which is remarkable for the fact that the studies of which it gives an account are the first in which the method of cutting sections has been applied to the examination of the very delicate and small eggs and embryos of Gastropod Molluscs. Dr. Bobretzky has also used and recommends a method which consists in hardening the embryo in dilute chromic acid and then observing it under the microscope as an opaque object.

Neither of these methods could be applied to all molluscan ova, for some are even smaller than those Dr. Bobretzky has had patience and skill enough to cut, whilst others are enclosed singly in large quantities of albuminous material from which they could not be removed in quantity either before or after the action of chromic acid. It is, therefore, fortunate that three genera have come to hand at Naples which could be treated in this way, and they have yielded in Dr. Bobretzky's hands results of the very greatest interest.

At the same time, whilst we feel bound to accept *pro tanto* the main facts with regard to *Nassa*, *Natica*, and *Fusus* which Dr. Bobretzky sets forth in drawings and letter-press, we are not on that account, disposed to follow him in generalizing his results so as to include in their dominion all the Gastropoda and even less all the Mollusca. In the study of embryology the practical lesson is daily being more and more forcibly impressed upon naturalists—that the assertion of generality throughout a class or phylum of organisms for a phenomenon observed only in two or three or even more members of that class or phylum is an exceedingly risky proceeding. Embryology in its present phase of development is full of surprises, and generalizations must be understood as possessing only the value—a very real and legitimate value—of hypotheses, which need again and again to be tested by further observation.

Dr. Bobretzky has been led to conclude from his observations that the mode of development observed by him in Nassa, Natica, and Fusus holds good for all the Mollusca, and since on an important point the facts observed by him differ from certain of those which I have observed and published in regard to other Mollusca, he does not hesitate to declare those particular observations of mine to be erroneous. His attempted generalization would have carried more conviction with it if he had been in a position to correct my statements by a study of the identical species—namely, *Paludina vivipara*, *Limnæus stagnalis*, and *Pisidium pusillum*, to which they had reference. I frankly admit that I have fallen into a similar error in suggesting, though I did this with some caution, that the facts observed by me in the above Molluscs have a wide significance and may be generalised so as to include not only the Mollusca but other animal groups in which the planula or gastrula develops by means of a blastopore.

The point as to which Dr. Bobretzky and I are at issue is this: Does the blastopore or gastrula's orifice of invagination in no case coincide with the mouth as I have maintained? Does it in no case among the Mollusca become the anus as Bobretzky has maintained? The truth appears to be that, as Bobretzky has shown, the blastopore or orifice of invagination of the gastrula *does* in certain cases among Mollusca coincide with the mouth, and on the other hand that it *does* in other cases coincide with the anus, as I have stated in Paludina and in Pisidium and as Agassiz established in certain Echinoderms; or it may coincide with neither mouth nor anus; or again, as I have pointed out in the case of Limnæus,[1] it may have an elongated form corresponding (in position though not in continuity) with the anus at one end and the mouth at the other.

Dr. Bobretzky having rejected my observations on the development of Paludina to which, as he rightly says, I attach most weight, and having expressed incredulity with reference to the two diagrammatic woodcuts which accompanied my note on the subject,[2] I give on the present occasion a more complete series of drawings illustrating the early stages of development of the Mollusc in question. Some of these drawings and notes I have had by me for some time, whilst others are due to observations made since Dr. Bobretzky's criticisms. I have to thank my friend Professor Lawson, of Oxford, for a copious supply of fine Paludinæ

[1] See this Journal, July, 1876.
[2] This Journal, April, 1875.

from the luxuriant old Botanick Garden of the University and for the use of his adjacent laboratory.

Paludina vivipara is so common a Gastropod and so convenient for embryological study that there can be little difficulty in confuting or confirming by actual study of the object in question statements made relating to its development. It would, however, be exceedingly difficult to obtain actual sections of its earlier phases; this is the less to be regretted since the young embryos are far more transparent than are those of most Gastropoda. The notion that only those embryos which admit of the section-cutting treatment can furnish really valuable evidence in embryological questions is a prejudice which needs to be checked at the present day. The embryologist who has not practised himself in the arts of hardening, embedding, slicing, and clarifying is prone to choose for his studies a certain class of embryos, namely, those which are transparent. Generally it may be said these are the developmental histories in which a small or unimportant amount of food-material (Nahrungsdotter) is present, and are consequently liable to certain common characteristics. On the other hand, the section-cutting embryologist is liable to occupy himself exclusively with the largest eggs which he can procure belonging to the particular animal tribe he wishes to investigate. These large eggs are as a rule eggs with a relatively large amount of food material, and present in their development characteristics dependent on this relatively large amount. Consequently, exclusive pursuit of the one method of study is as likely as the other to give results which are arbitrarily though unconsciously "selected."

The embryologist has to be on his guard lest his favourite method of manipulation distort his view of the facts in the one or the other direction.

Dr. Franz Leydig in one of his classical monographs published as long ago as 1850 ('Zeitschrift fur wiss. Zoologie,' vol. 2) gives an account of the anatomy and development of Paludina vivipara. Nothing has been published in the past twenty-six years relative to the development of that very common Gastropod except my own note in this Journal in April, 1875. Leydig's description and figures of the earlier stages of the development are correct so far as relates to the actual appearances; but the use of higher powers of the microscope than those which were available so long since renders it easy to make out at the present day many details as to the arrangement of the cell-elements of the embryo

which are important in modern embryology, and shows that the orifice which he took for the first indication of the mouth is what we now know as the orifice of invagination or blastopore which can be traced in subsequent development *not* to the mouth, but to the anus.

Stage 1.—From the early spring until the middle of October large specimens of Paludina contain in the upper part of the oviduct ova which are in the condition of simple unsegmented corpuscles such as that represented in Plate XXV, fig. 1. Each egg in Paludina is contained when in the oviduct, in a relatively very large envelope distended by a thick but transparent albuminous fluid in which the egg-corpuscle floats and from which it cannot be entirely liberated.

The egg-envelopes lying somewhat nearer the mouth of the oviduct contain embryos in various stages of development, so that it is possible from one specimen to obtain a very complete series. The youngest oviducal eggs such as that represented in Plate XXV, fig. 1, present in the living condition no indication of nucleus, of germinal vesicle or germinal spot. The egg-protoplasm at its periphery appears to be hyaline and highly refracting, whilst it is charged with fine granular matter beneath the surface and with minute spherical particles of a bright yellow colour which give the whole egg an orange-brown tint.

Stage 2.—The ova in the various stages of division may be found in one or another specimen. I have selected for fig. 2 an embryo in which the cells are still of large size. They appear all to share equally the possession of the finely granular and yellow coloured food material and to present no grouping into a hemisphere of smaller and a hemisphere of larger cells. Hence the "polyplast" or "morula" as Haeckel has termed it, is of the simplest type—an "archimorula."

Stage 3.—In fig. 3 an embryo is represented in which the division of the embryonic cells has proceeded further, whilst a central space or "cleavage cavity" has been formed. The opacity of the embryo does not permit one to make out very satisfactorily the size of this cavity, and whether the spherical form is really possessed by the "blastula" stage as this one-cell-layered vesicle is called in Haeckel's terminology. Possibly a slight cup-shaped depression already extends over one pole of the blastula.

Stage 4.—In figs. 4 and 5 are reproduced sketches taken from living specimens showing the formation of the endoderm by invagination. The ectodermal cells are seen to be somewhat columnar and but little charged with granular matter, though they retain to a late period some of the

yellow spherical particles which were noticed in the original egg-cell. The endodermal cells are more swollen and globular and contain so much of the finely granular and yellow-coloured food material as to render them nearly opaque and of a dark brown tint in the mass. The commencing invagination is limited by a circular margin (fig. 5, below), which remains throughout the subsequent stages here figured very distinct. The circular form of the blastopore and the completely pyramidal form of the embryo at the period of its full development (fig. 5) are worthy of especial note. In the finished gastrula of Paludina the circular blastopore occupies the base of the cone directly opposite to the apex. It has not, as it has in Limnæus and in Polycera, an eccentric position nor an elongated form.

Stage 5.—A slight change of form marks the next stage of development. The blastopore narrows a little, whilst the opposite region of the embryo becomes marked by a ciliated girdle (figs. 7 and 8). This is the trochosphere stage. The ciliated girdle is placed well forward in Paludina, so that it does not, as in some Mollusca and many Vermes, take an actually equatorial position. Very rapidly now the embryo increases in size and the anterior field or upper hemisphere of the trochosphere (the area of the velum) dwindles as compared with the posterior field or lower hemisphere.

With the first changes of shape which mark the development of the ciliated girdle and the assumption of the trochosphere stage, certain changes in the cells of endoderm and ectoderm are to be detected. Both become more transparent and those of the endoderm have diminished in size, whilst a space separating endoderm and ectoderm *traversed by delicate protoplasmic filaments* and not to be confounded with the original cleavage-cavity of the blastula (fig. 4, *sc*) is visible. This new cavity is the mesoblastic cavity or cœlom. The filaments which pass across it from endoderm to ectoderm are the first indications of the branched and fusiform corpuscles which subsequently develope in large quantity on its walls and lay the foundations of muscular and connective tissue and hæmolymph. I have described a similar arrangement of the commencing mesoderm in Limnæus. At this period of development it is perfectly easy to satisfactorily determine that the only direct continuity of endoderm and ectoderm is at the blastopore, and that this is separated from the position subsequently occupied by the mouth—by the rudiment of the foot (fig. 8).

Stage 6.—The embryo increases in size and the blastopore narrows without any trace of the mouth being visible ex-

cepting a large superficial concavity at the point in front of the velum, where it is destined definitely to make its appearance. At this stage, represented in figs. 10, 11, and 12, the foot has pushed itself forward on that aspect of the embryo which may be called the pedal or ventral, and towards which the blastopore faces. The blastopore has a very distinct margin of epidermic cells, and is at this period ciliated. This up-standing collar of ectodermal cells may perhaps be taken to represent the proper anal invagination or "proctodæum" (so-called to balance the "stomodæum" or oral invagination) which in Paludina absolutely coincides with and is one with the blastopore. Thus "the rectal peduncle" or "pedicle of invagination," as I have termed the narrow part of the endodermal invagination nearest to the blastopore, remains permanently open to the exterior. In Limnæus it is closed up at an early period by the growing together of the lips of the elongated blastopore at its posterior or anal end.

In Pisidium also it is closed at a very early period by the entire obliteration of the blastopore. In both these genera the "pedicle of invagination" is subsequently eaten into by a new invagination of the ectoderm of small extent in these Molluscs, but apparently capable of large development in other classes of animals (e. g., Arthropods) which may conveniently be termed the "proctodæum." The "proctodæal tract" of the alimentary canal, derived as it is from a second invagination of ectoderm, must be distinguished (where it is developed to an appreciable extent) from those tracts derived from the archenteron or endoderm of the gastrula; just as the stomodæal tract derived from the oral invagination and sometimes loosely spoken of as pharynx, must be distinguished from the archenteron. When the blastopore remains open throughout development the proctodæum, though reduced in size, can still be followed as an upgrowth around the blastopore, and similarly it appears from Bobretzky's observations that the strangely opposed case of the coincidence of blastopore and mouth does not prevent the development of the stomodæum as a new growth which has either the form of an upgrowth around the sunken blastopore as in Fusus (Bobretzky's Plate xii,) or as a cæcal ingrowth at the point of closure of the blastopore as in Natica (Bobretzky's Plate x).

The form which the embryo has now attained is such that under a slight pressure it readily takes up one of the two positions drawn in figs. 10 and 11—either presenting to you the dorsal pallial surface or the ventral pedal surface. When

the dorsal surface is presented a large portion of the velar area—that area enclosed by the ciliated ridge of the velum—is visible, since the velar area forms a plane having a dorsal rather than a polar aspect, as may be best seen in the lateral view (fig. 12). In the centre of the pallial region is a small strongly marked pit, from which the cells of the epidermis are seen to radiate, each cell having an elongated form; the area occupied by these elongated radiating cells is sharply marked off and is of a circular form (fig. 10.) This area, with its central groove or cup, is the structure which I discovered in the Lamellibranch Pisidium and in the Gastropod Aplysia in 1871, and subsequently in the 'Annals and Magazine of Natural History' for February, 1873, described as the "shell-gland," and further in this Journal, in 1874, showed to be an organ common to the various classes of Mollusca either in their adult or embryonic conditions.[1] Its occurrence has since been noted by other observers. In Paludina, when the shell has already assumed a dome-like form, the pit of the shell-gland is still in existence and is filled by a small chitinous knob of the shell.

In fig. 11 the same embryo as that seen dorsally in fig. 10 is represented as seen from the pedal or ventral aspect. The anterior margin as this view is formed by the anterior border of the velum. It is just below this at the spot marked *sm* that the mouth *subsequently* makes its appearance. At this stage there is here no trace of mouth or stomodæum—no thickening of the ectoderm or special connection between it and the close well-rounded upper end of the archenteron or endodermal sac. In specimens of a very little later growth than this a broad shallow depression can be detected between the margin of the velum and the protuberance of the foot at the spot marked *sm* in figs. 11 and 12, and it is thus as shown by the appearance of still further developed embryos such as fig. 14 represents—that the mouth and stomodæum make their appearance. The woodcut (figure 2) in my paper in this journal, April, 1875, represents an intermediate stage.

The blastopore is seen to lie on the ventral surface, in the stage represented in figs. 10, 11, and 12, a position to which it tends from the earliest assumption of the Trochosphere phase of development (see figs. 7, 8, and 9).

Up to this period the invaginated archenteron (primitive or gastrula stomach) has retained a simple oval form narrowing to a short neck which opens by the blastopore: it

[1] In the Proc. Royal Society, March, 1874, I mentioned that I had found the embryonic shell-gland in the following genera, Pisidium, Aphysia, Neritina, Limnæus.

does not exhibit the bilobed character which is so early assumed by the archenteron of Pisidium and of Limnæus.

Stage 7.—In figs. 13, 14, and 16 an embryo is represented which has grown to double the size of that drawn in figs. 10, 11, 12, and has not only relatively diminished the size of its velum and increased the size of its foot, but has also developed a mouth and stomodæum. This stage corresponds to the well-known Veliger of other Gastropod ontogenies, and by the development of the shell and pallial region, by the further increase of the foot, reduction of the velum and growth of tentacles, the embryo passes from it to the adult form, as may be well seen in Leydig's excellent figures.

The development of the nervous system, organs of sense, and of the parts and appendages of the alimentary canal to which the archenteron gives rise as well as the ovary and testis, require further study than I have yet given them in this Mollusc. The stage to which figures 13 and 14 bring the development are sufficient for my present purpose, which is to demonstrate that the blastopore is coincident with the anus and that the mouth develops quite independently and comparatively late.

In the embryo drawn in fig. 14 the same parts are present as in that represented in fig. 12, the larger size of the foot and the smaller size of the velum being the most striking changes of their common parts. But in the later embryo we find in addition to this that the upper end of the archenteron has become bifid, and received into its grooved extremity a deep and wide invagination of the ectoderm, which is plainly enough the stomodæum, or embryonic pharynx. Of this structure there is no trace in the younger phase, and there can be no possible doubt of its entire independence of the blastopore. Equally impossible is it to doubt that the blastopore has all along persisted as the one orifice of the archenteron situated near the posterior pole of the embryo, altogether remote from the site of the mouth.

In the embryo of fig. 14 the tissue between ectoderm and endoderm has increased in abundance, consisting now of numerous fusiform cells, some of which have two delicate branches at one pole. The cœlomar surface of both the ectodermal and endodermal cells is also now closely invested by cells similar to the delicate up-standing fusiform corpuscles, which stretch across the cœlom or body cavity. These investing tunics of mesoderm or mesoblastic tissue (the hypoderic and hypenteric cell-layers) consist of delicate flattened corpuscles which are connected by their processes with the traversing fusiform corpuscles.

Conclusions.—The facts in the development of Paludina which I have now placed before the reader by word and by carefully executed drawings do not leave the possibility of a doubt that in this particular Gastropod the blastopore coincides with the anus and that the mouth is a remote and entirely independent formation. Such being the case the observations of Bobretzky to the effect that, in three allied Gastropods whose eggs contain copious food-material, the blastopore coincides with the mouth—cannot be held to have a general import. The fact must be admitted, improbable as it had seemed both to Bobretzky and to myself, that there is *not only one* possible fate for the blastopore of the Molluscs (or we may suppose of other Metazoa) in cases where it does not simply close and leave no trace. It appears that the *anal* invagination or proctodæum is sometimes formed at the very spot where the blastopore closes or even before it closes, and on the other hand the *oral* invagination or stomodæum is sometimes formed at that spot. In fact, there is no necessary connection between the blastopore and either the mouth or the anus; sometimes they are all three independent, sometimes the former coincides with one of the two latter, and sometimes, being elongated in shape, coincides with both.

Dr. Bobretzky's hazardous process of reasoning, by which he was led to deny the correctness of my account of the "pedicle of invagination" and development of the mouth and anus in *Pisidium* ('Phil. Trans.,' 1875), as well as in *Limnæus* and *Paludina*, consisted in the inference of *generality* for the phenomena observed by him in the particular cases of *Nassa*, *Natica*, and *Fusus*. On bringing this inference to the test of facts, it is found to be devoid of justification, since in involves the contradiction of published observations beyond Dr. Bobretzky's experience—which observations are and can readily be shown to be, correct. Though I am thus brought into collision with the Russian author in matters of inference, I wish to express my high appreciation of the value of his work, the difficulty of accomplishing which and the consequent skill and courage of the author none can estimate so well as those who have occupied themselves with the same material of research.

Note *on the* Lymphatics *of* Mucous Glands. By P. Kidd, B.A. Oxon. (With Plate XXVI.)

The subject of this investigation was the mucous glands of the pharynx and œsophagus in the dog. Before proceeding to describe our results we shall shortly state what was previously known on this point.

Teichmann 'Das Saugadersystem,' has described the minute lymphatics of the pharynx and œsophagus, and subquent workers on the anatomy of these parts quote Teichmann as their authority on this point.

Taking Teichmann, therefore, as the standard authority, we find it stated that in the œsophagus the lymph-capillaries form networks whose branchlets take a longitudinal course.

Teichmann then goes on to say that in the case of the pharynx, œsophagus, and trachea, the mucous glands and the lymph-capillaries stand in no direct relation.

We made several injections of the pharynx and œsophagus of dogs recently killed, by the method of puncture. The injections used were Berlin blue and nitrate of silver solution.

The result of our injections of the œsophagus and pharynx agree closely with Teichmann's statements, and we give a drawing (Pl. XXVI, fig. 1) of the rough surface-appearance of the injected lymph-capillaries of the pharynx. It will be seen by referring to the drawing that the network which the lymph-capillaries form in the superficial part of the pharynx is very close.

In the case of the mucous glands our results differ from those of Teichmann, for we find in specimens injected with Berlin blue that the mucous glands lying in the submucosa of the pharynx are provided with a special system of lymph-vessels. A drawing (fig. 2,) is given of one specimen obtained by this method.

In this as in other instances the mucous glands were seen to be surrounded by broad bands of injection which give off branches here and there. Some of these branches penetrate between the lobules of the glands and follow the individual lobules more or less closely.

The broad bands of injection represent large lymph-vessels, which form tubes round the mucous glands and give off smaller vessels which follow the course of the individual lobules. In some cases the connection between the glands and lymph-vessels is less close, the glands being loosely surrounded by a large lymphatic space or sac.

In such a case the gland seems as if invaginated in a capacious lymphatic sinus, no special offshoots of the main vessel running in between the lobules of the gland (fig. 3).

In the case of the nitrate of silver injection we were less successful, though finally we obtained evidence of the presence of endothelial outlines round the glands. Owing to the extreme delicacy of these endothelial membranes the chances were small of obtaining a section in the plane of the membranes which should give a surface-view of the silver-stained endothelium.

Although thick sections were made to increase the chances of getting such a view, no very characteristic appearances were obtained. But large lymph-vessels were seen in close proximity to the glands, and in some cases fine endothelial markings were noticed here and there around the lobules. Sections of an uninjected dog's pharynx hardened in a mixture consisting of chromic acid ¼ per cent. 2 parts, and spirit 1 part, confirmed the results of our injections.

Here the mucous glands were seen to be surrounded by larger or smaller spaces limited by fine lines in which were set oblong nuclei at regular intervals, reminding one exactly of the profile appearance of an endothelial membrane. Here also the distribution of these lymph-vessels or spaces varied somewhat in different groups of glands. In some the lymph-vessel was continued into the interior of the gland around individual lobules, in others the lymphatic sheath was thrown loosely around the entire gland. In some cases the ducts of the glands were also provided with a sheath continuous with that surrounding the lobules.

A similar arrangement prevailed in the mucous glands of the œsophagus.

Teichmann moreover denies that the mucous glands of the tongue have any lymph-vessels. Here again our results are at variance with his. The appearances observed on the tongue resemble closely those seen in the pharynx, the mucous glands of the tongue being provided with similar encircling spaces lined by endothelial membranes with regularly disposed oblong nuclei.

We have not investigated other mucous glands than those here specified, but it would seem that in all probability a similar arrangement of the lymph-vessels obtains in all mucous glands. This would be in accordance with Leopold's researches on the glands of the uterus, which he finds are invaginated in lymphatic sheaths. ("Die Lymphgefasse des normalen nicht schwangeren Uterus," 'Arch. f. Gynaekologie,' Bd. vi). Moreover, this would also fit in with Gianuzzi's inves-

tigations on the salivary glands ('Bericht der Sächs. Ges. der Wiss.; Math. Phys. Cl.,' 1875, and 'Centralblatt,' 1875).

It may be also mentioned that between the individual muscular bundles of the pharynx there may be seen fine intermuscular lymph-spaces lined by endothelial membranes, an arrangement analogous to the interfascicular lymph-system in connective tissue.[1]

I have to thank Dr. Klein, under whose direction these investigations were made, for his advice and assistance.

Some Recent Views *as to the* Composition *of the* Fibro-vascular Bundles *of* Plants.

By Sydney H. Vines, B.A., B.Sc., Fellow of Christ's College, Cambridge.

(With Plate XXVII.)

In the consideration of this subject the first important point is to distinguish with precision between the elements which make up the fibro-vascular bundles and those which belong to the ground tissue in which the bundles are arranged. In those plants in which completely developed fibro-vascular bundles first occur the limit between the fibro-vascular and the ground tissues is clearly defined by means of one or more layers of cells which form a sheath for the bundle (Kritenchyma or Koleochyma[2]). In a fern, for instance, such as *Pteris aquilina* (Fig. 1, page 389), we find each fibro-vascular bundle invested by a sheath of two layers of cells, and very frequently it may be observed that the cells of the ground tissue which are adjacent to these sheathing cells are of a brown colour, have thickened walls and an elongated form. To a mass of these thick-walled cells the name Sclerenchyma has been given.

It is evident that of the two layers of sheathing cells the outer (*a*) forms the limit of the fibro-vascular bundle, and it is rendered conspicuous by the absence of starch from its

[1] The arrangement of lymph-spaces in bundles of striped muscle-fibres in the tongue, pharynx, and other organs, is precisely the same as that described by Axel Key and Retzius in nerve-bundles, viz. lymph-spaces of the perimidium are in continuity with spaces lying between smaller groups of muscle-fibres and penetrating even between individual fibres. The spaces are lined by connective-tissue cell-plates.

[2] Russow, 'Vergleichende Untersuchungen, Mem. de l'Acad. Imp. de St. Petersbourg,' t. xix, No. 1, p. 168, 1872. Also, idem, 'Betrachtungen über Leitbündel u. Grundgewebe,' Dorpat, 1875, p. 71.

cells. This layer is the true sheath of the bundle, and has been particularised under various names (Schutzscheide,[1]

FIG. 1.—Transverse section of bundle of *Pteris aquilina* (after Sachs). *X*, xylem; *P*, phloëm. *a*, bundle-sheath; *b*, phloëm-sheath. *G*, ground tissue.

Vaginalschicht,[2] &c.); but we shall always refer to it as the "bundle sheath." The inner layer (*b*) consists of parenchymatous cells like those of the outer layer, but differing from them in that they contain starch, and to this layer Russow[3] has given the name of Phloëm sheath. These two layers, then, together make up the true sheath tissue or koleochyma of the bundles of this fern. When the bundle sheath is invested by sclerenchymatous cells we may regard these cells as forming yet another covering for the bundle, and to this the name of "external sheath" may be applied (Aussenscheide, Russow).

In Lycopodium (chamæcyparissus) we meet with these same layers, but their arrangement has been modified as a consequence of the fusion of the fibro-vascular bundles to form an axial cylinder. Here the bundle sheath, which is very well marked, surrounds the whole of the vascular tissue, from which it is separated by a phloëm sheath (Hegelmaier),

[1] Caspary, 'Jahrb. f. Wiss. Bot.,' i and iv.
[2] Sachs, 'Lehrbuch der Botanik,' 4te Aufl., p. 126.
[3] 'Vergl. Unters.'

consisting, not as in Pteris, of a single layer of cells, but of several layers, and the external sheath also consists of numerous layers.

In Selaginella (inæqualifolia), in the stem of which, as in that of Pteris, the bundles are isolated, each bundle is surrounded by a cavity, its connection with the ground tissue being maintained by bridles of cells, which reach from the one to the other. The bundle is bounded towards the air cavity by a definite layer of cells, which is apparently the bundle sheath. Russow[1] regards the lacunar tissue as representing this structure, but the previous view seems to be probably the correct one, when a comparison is made of the bundle of Selaginella with that of the fern and of Lycopodium. Within the bundle sheath we find a phloëm sheath, consisting, as in Lycopodium, of several layers of cells. The external sheath may, perhaps, be represented here by the lacunar tissue and by the well-defined layer of cells which bounds the air cavity on the side of the ground tissue.

In the stems, rhizomes, and roots of many Monocotyledons an evident bundle sheath is to be found (Pl. XXVII, fig. 1). It is especially constant in the Cyperaceæ and Juncaceæ, and often occurs in Gramineæ. We find no structures in the bundles of the Phanerogamia which correspond to the phloëm sheath[2] in the vascular Cryptogams, but the external sheath is often well developed. In fig. 1 we see a dense mass of cells (bast fibres) investing nearly the whole of the circumference of a fibro-vascular bundle, and evidently corresponding to the structure referred to in the Fern. In those Monocotyledons which do not possess a well-marked bundle sheath it seems difficult to decide whether these bast fibres belong to an external sheath, or whether they are elements of the fibro-vascular tissue. To this difficulty we shall hereafter refer.

A bundle sheath occurs in the bundles of the roots of the great majority of the Dicotyledons, and of all Gymnosperms, and of the stems of many Dicotyledons (Campanulaceæ, Primulaceæ, &c.), which invests the whole of the vascular bundles.[3]

In some Dicotyledons each bundle has its own sheath, as in *Eranthis hyemalis*, *Ranunculus lingua* and *flammula* (Pl. XXVII, fig. 2).

An external sheath occurs in the bundles of the roots of

[1] 'Betrachtungen,' p. 76.

[2] Perhaps we may regard the colourless parenchyma investing the bundles in the leaves of Conifers as constituting a phloëm-sheath.

[3] Pleromscheide (Sachs), Gemeinsame Scheide (Russow).

many Dicotyledons (Pomeæ, Papilioneæ, &c.), and in those of all Conifers, except Ephedra and the Abietineæ.[1]

It is interesting to find that, in Dicotyledons, in which growth in thickness continues, the cells of the bundle sheath very rarely become thickened (except roots of *Ranunculus acer*, *chius*, *lingua*, and others), forming the "Steifungscheide" of Russow, whereas in Monocotyledons this frequently takes place in the rhizomes and roots.

We have now briefly traced the range and distribution of these sheathing cells, and it remains for us to consider what is their morphological value. As regards the external sheath, there can be no doubt that its elements are extremely modified forms of the cells of the ground tissue. Russow[2] refers the bundle sheath and the phloëm sheath, in fact, his "Koleochyma," as well as the external sheath, to the ground tissue, and herein he is followed by Sachs.[3] Falkenberg,[4] however, cannot accept this view in its entirety. He mentions that, in Hedychium and Canna, when some of the bundles bend outwards into the leaves they lose their true vascular elements, and consist only of bast fibres; and so, on Russow's view, we would have bundles which terminated blindly at their upper extremities, their sheaths alone forming their continuation. He goes on to say that such an independent origin of sheaths is an impossibility, unless the distinction between the vascular and ground tissues be given up. He concludes that so long as this distinction remains we must consider the sheaths of Monocotyledons at least as belonging in some cases to the ground tissue, in others to the vascular tissue. On the whole, however, the balance of evidence seems to incline towards Russow's view, and it is to that view that reference will be made throughout this paper. We may now go on to consider the composition of the fibro-vascular bundle itself.

In the year 1858 Nägeli published a work on this subject,[5] in which, for the first time, a definite and satisfactory account was given of the structures in question. He regarded the fibro-vascular bundles of Dicotyledonous plants as consisting of two principal parts, an outer and an inner, separated by the layers of delicate cells forming the cambium, the outer

[1] Strassburger, 'Die Coniferen u. Gnetaceen,' 1872, p. 346. He terms this structure "aeussere Schutzscheide."

[2] 'Vergl. Untersuch.,' p. 166.

[3] Loc. cit., p. 126.

[4] 'Vergl. Unters. ueb. d. Bau d. Vegetationsorgane,' 1876, p. 142.

[5] 'Beiträge zur Wissenschaftlichen Botanik,' Heft i, von Carl Nägeli, Prof. in München.

being the bast or phloëm, the inner the wood or xylem. The phloëm he found to be made up of parenchyma, of liber cells or bast fibres, of soft bast, and of latticed cells; the wood, of vessels, of woody fibres, and of parenchyma. He explained the structure of the fibro-vascular bundles of Monocotyledonous plants in essentially the same way. He found that here also they consisted of an inner xylem composed of vessels and woody cells, and of an outer phloëm composed of bast fibres. He found that they were separated, not, indeed, by layers of cells capable of undergoing division constituting a cambium, but by an aggregation of delicate cells, to which he gave the name of cambiform. Moreover, in the bundles of these plants, this separation of the xylem from the phloëm by the intervention of the cambiform tissue is confined only to the central part of the bundle; towards its circumference, on each side, the elements of the xylem and of the phloëm imperceptibly blend (fig. 4).

These views of the general structure of fibro-vascular bundles met with very general acceptance, and, with some modifications, are still received as an adequate expression of our knowledge of the subject. According to a recent exposition of them,[1] we find that the terms xylem and phloëm are retained, and are applied in the same sense in which Nägeli first employed them, but that, as the result of subsequent investigations, the account now given of the histological elements of which the phloëm and the xylem respectively consist, differs somewhat from that given by Nägeli.

Sachs states that both the xylem and the phloëm consist of three forms of tissue: (1) combinations of cells to form vessels (vessels of the xylem, sieve tubes of the phloëm); (2) prosenchymatous cells (woody fibres of the xylem, bast fibres or hard bast of the phloëm); and (3) parenchymatous cells (wood parenchyma of the xylem, soft bast or bast parenchyma of the phloëm).

In comparing these two accounts we find that the vascular elements of the xylem are similarly described in each, whereas those of the phloëm are termed, in the one latticed cells, in the other sieve tubes. This difference of name is due to the fact that it is not always easy to detect the perforations in the septa between the cells which are characteristic of sieve tubes, and serve to distinguish them from the latticed cells, the cavities of which are not in communication. With regard to the prosenchymatous elements, we find a complete agreement, and this is the case also with

[1] Sachs, loc. cit., p. 112.

the parenchymatous, with this single exception, that Nägeli speaks of parenchyma, other than the soft bast, as existing in the phloëm, whereas Sachs makes no mention of it. We have yet to notice what is the current view of the nature of the mass of tissue occurring in the bundles of Monocotyledons termed by Nägeli cambiform, and which he described as separating the xylem from the phloëm. This cambiform tissue is now regarded as being the equivalent of the soft bast of the bundles of Dicotyledonous plants.

The elements comprising the fibro-vascular bundles of the vascular Cryptogams may also be classified under the heads of vascular tissue, prosenchyma, and parenchyma; but their occurrence is not so constant as in the Phanerogams.

In the stem of Equisetum the xylem consists of parenchyma and of vessels, and the phloëm of parenchyma, bast fibres, and vessels (sieve tubes). The bundles of the stem of Lycopodium differ in histological structure from those of Equisetum, in that here the xylem consists only of vessels which are no longer separated by parenchymatous cells (geleitzellen of Russow), whereas the phloëm presents three characteristic elements. The elements present in the bundles of *Pteris aquilina* are the same as those occurring in Equisetum, and this is true also of Marsilea, but in this case the parenchymatous cells of the xylem (geleitzellen) are very few.

The grounds upon which certain of the elements of fibro-vascular bundles are referred to the phloëm or to the xylem deserve consideration. In the case of Dicotyledons it is evident that all those elements of the bundle which are external to the cambium layer belong to the phloëm, whereas those which are internal to it belong to the xylem, and Schwendener is, perhaps, right in saying[1] that Nägeli had this relation of the inner and outer parts of the bundle to the cambium in view when he first suggested the use of the terms. But the distinction of these two parts of a bundle from one another is by no means so simple when the bundle belongs either to a Monocotyledonous or to a Cryptogamous plant. In such a case a decision can only be arrived at by detecting characteristic histological elements, such as the vessels, in different parts of the bundle. The vessels of the phloëm and those of the xylem, however, are generally separated by parenchymatous cells, and these present no such difference as would lead to any definition of one part of the bundle from the other. Russow[2] has endeavoured to remove this difficulty by regarding the parenchyma of both phloëm

[1] Loc. cit., p. 4.

[2] 'Betrachtungen,' p. 17.

and xylem as forming a common continuous tissue of similar cells (Geleitzellen or Leitzellen), which invest the vessels of the bundle.

Now that we have gained some definite idea of the meaning of the words "sheath" and "fibro-vascular bundle," we may go on to inquire into the relations existing between them.

Taking the fern (*Pteris aquilina*, fig. 1, p. 389) as an example of the vascular Cryptogams, we find that here the relations between sheath and bundle are of the simplest. In the central portion of the bundle we have the xylem, around it we have the layers of phloëm, and investing these we have the two layers of sheathing cells.

Passing on to a similar consideration of the bundle in those Monocotyledons, the stems of which do not increase in diameter, we find a xylem forming the inner part of it, a soft bast lying externally to this, and then a mass of thick-walled prosenchymatous cells quite at the exterior, which invests more or less of the circumference of the bundle (Pl. XXVII, fig. 3). Nägeli regards these cells as forming the true bast portion of the bundle. Dippel[1] considers that those cells which lie on the peripheral side of the bundle are bast fibres, whereas those which lie on its central side are woody fibres, and this view coincides with those of Unger[2] and of Schacht.[3] But on referring to the account given of the bundle sheath we find that these cells have been claimed as belonging to the ground tissue. Von Mohl,[4] as early as 1833, doubted the correctness of the view that all prosenchymatous cells belonged to fibro-vascular bundles. He says: "We are accustomed, in Phanerogams, to meet with thick-walled prosenchymatous cells only in the fibro-vascular bundles, and we allow ourselves, therefore, to be easily led into regarding every aggregation of elongated cells as either wood or bast," and in his account of the structure of the palm stem,[5] speaking of this thick-walled prosenchyma, he goes on to say, in opposition to Link[6] and to Kieser,[7] that these cells, in their thick walls, their small width, and their elongated form, resemble bast cells, but that it would be a grave error to compare this mass of tissue with the bast of Dicotyledons. This latter erroneous view he supports by several arguments, and he goes on

[1] 'Das Mikroskop.,' ii, p. 210, 1869.
[2] 'Anat. u. Phys. d. Pflanzen,' p. 218, 1855.
[3] 'Grundriss der Anat. u. Phys.,' p. 64, 1859.
[4] 'Vermischte Schriften,' p. 116, 1845.
[5] Loc. cit., p. 150.
[6] 'Grundlehren,' p. 143, 1807.
[7] 'Grundzüge der Anat.,' p. 72, 1815.

to prove that these so-called bast fibres of Monocotyledons belong in reality to the ground tissue.

Mohl's view was supported and further developed by Schultz-Schultzenstein,[1] who went so far as to suggest that since the bast fibres were a highly differentiated form of ground tissue, they ought, therefore, to be no longer regarded as belonging to the fibro-vascular system. Russow[2] regards these prosenchymatous cells as sheath cells belonging to the ground tissue. Schwendener[3] has demonstrated the correctness of the views of his predecessors, and has founded on them a new theory of the general composition of fibro-vascular bundles. He brings forward examples to show that this bast tissue bears no definite morphological relation to the other elements of the vascular bundles, and he points out that a well-marked layer (bast sheath) often intervenes (in all Cyperaceæ and Juncaceæ and in many Gramineæ, Pl. XXVII, fig. 1) between the bast and the vascular bundle, consisting of cells, the walls of which are generally somewhat thickened and resist the action of concentrated sulphuric acid, thereby indicating that they can be only very slightly permeable by water. These cells differ from the bast cells in the different form and arrangement of the pores in their walls, and frequently in that they are parenchymatous. To the mass of tissue lying within this layer, which is, in fact, a fibro-vascular bundle without any hard bast, he gives the name of Mestom. He finds, further, that thick-walled cells, similar to the bast cells, occur in the cambiform tissue in various palms, Liliaceæ and species of Pandanus, and in the ground tissue of some Cyperaceæ and Gramineæ. From these facts, and from similar observations upon vascular Cryptogams and Dicotyledons, which we have yet to consider, he is led to the conclusion that these bast cells are arranged in obedience to mechanical and not to morphological laws; that they may be developed, in fact, in any tissue which requires support.

It has already been said that in connection with the secondary fibro-vascular bundles of those Monocotyledons which grow in thickness, we find cells occupying that position in relation to the Mestom which is occupied by the bast cells in other Monocotyledons, from which they differ in structure (fig. 4). Schwendener regards these pitted cells as being the analogues of the bast cells, whereas Russow[4] con-

[1] 'Cyklose des Lebenssaftes,' 1841, p. 245.
[2] Loc. cit., p. 171.
[3] 'Das Mech. Prinzip.,' p. 16.
[4] 'Betrachtungen,' p. 6.

siders them to be elements of the xylem. It seems probable that the view which Schwendener takes of their morphological value is the correct one, and, if this be so, all that has just been said of the bast cells applies equally to these.

With regard to Dicotyledons and Gymnosperms we have seen (fig. 2) that the bast cells, which we no longer consider as essential constituents of the phloëm, most frequently invest the outer surface of the soft blast (true phloëm) as a supporting sheath, but, as in the Monocotyledons, they bear no constant relation to the other elements of the bundles. We find them alternating with layers of soft bast (Tilia, Juniperus, fig. 5), we find them lying internally to the xylem (Cucurbita, Bryonia, Fig. 6), and we find them forming independent bands in Rhipsalideæ.[1] Schwendener[2] goes on to point out that there is a similarity, amounting almost to identity, between the structure of the bast cells and that of the woody fibres (libriform of Sanio[3]); and he regards these structures as being intimately related. He concludes that, as in Monocotyledons so in Dicotyledons, the prosenchymatous cells are arranged in the tissues in obedience to mechanical and not to morphological laws.

As the result of his investigations, Schwendener is led to regard all the prosenchymatous cells of a plant, whether occurring in the ground tissue, in the phloëm, or in the xylem, as being specifically mechanical elements, and as forming parts of the hard skeleton of the plant, arranged in accordance with purely mechanical laws. For the entire system made up of these cells he proposes the name "Stereom," each one of the cells being termed a "sterëide," but these cells are still called bast cells, even by himself.

These views have been, on the whole, accepted as well founded by subsequent observers. Russow[4] admits that the view which regards the bast cells (sclerenchyma), which more or less invest the periphery of the bundles, as belonging, not to the bundles, but to the ground tissue, is proved as well by morphological and physiological as by phylogenetic evidence, for he thinks it probable that the function of support which, in the Archegoniata, is assigned to elements of the ground tissue may, in course of time, have been transferred, by gradual adaptation, to the elements of the bundles.

[1] Vöchting, "Beit. zur Morph. u. Anat. der Rhipsalideen," 'Jhrb. f. Wiss. Bot.,' ix, p. 475, 1873-74.
[2] Loc. cit., p. 6.
[3] 'Bot. Zeitung,' 1864-65.
[4] 'Betrachtungen,' p. 12.

Falkenberg[1] considers, with reference to Monocotyledons, that by the history of their development, by the fact that all intermediate forms are to be found between the ordinary parenchymatous cell of the ground tissue and the prosenchymatous bast cell, it is clearly shown that the bast cells are to be referred to the ground tissue, and he also agrees that these cells are arranged in accordance with the laws of mechanics.

It will be interesting, in conclusion, to see how these views affect our conceptions of the ground tissue and of the fibro-vascular bundle. Before their publication Russow[2] had formed, from morphological considerations, much the same conclusions as to the limits between the ground and vascular tissues as Schwendener formed from physiological. Some of his conclusions were criticised by Vöchting,[3] and were regarded by him as obliterating all distinction between the tissues in question. Russow,[4] however, points out that the formation of the bast, in Monocotyledons, for example, and more especially of that portion of it which is closely applied to the bundles, must necessarily take place simultaneously with that of the vascular elements themselves, in order to afford protection and support to the young and tender tissues; and he argues that Vöchting is incorrect in regarding the bast cells thus formed as belonging to the vascular bundles, and that this simultaneous origin from apparently the same primitive tissue is insufficient evidence of morphological equivalency.

As to the fibro-vascular bundles themselves, we can regard only the vessels of the phloëm and of the xylem, together with the parenchymatous cells which accompany them (Geleitzellen), as being their essential morphological elements; and herein we follow the view of Schwendener—a view which is also accepted by Falkenberg.[5]

When prosenchymatous cells occur in a bundle we must regard them as elements of the bundle, but only as comparatively accidental elements, since their presence there is in accordance, not with the morphological principles which regulate the arrangement of the parenchymatous cells in connection with the vessels, but with principles of a purely mechanical sort.[6]

[1] Loc. cit., 1876, p. 138 and 158.
[2] 'Vergl. Untersuch.'
[3] Loc. cit., p. 475.
[4] 'Betrachtungen,' p. 14.
[5] Loc. cit., p. 155.
[6] Schwendener, loc. cit., p. 2. In his work these mechanical laws are developed at length.

We have yet to draw a distinction between the prosenchymatous cells (stereïdes) which belong to the bundles and those which belong to the ground tissue, by means of which we may be able to refer any stereïde to its proper morphological position. Such a difficulty does not arise with reference to the woody fibres, or in the case of certain of the forms of prosenchyma occurring in the ground tissue (Hypoderma, Collenchyma)—their position is generally sufficient to indicate their real nature—but it exists especially in the case of the bast fibres. An investigation of their structure gives no characteristic which might serve to distinguish the bast fibres which belong to the ground tissue (external sheath) from those which belong to the bundles (hard bast). We must remember, however, that those cells which form the external sheath are necessarily outside the bundle sheath, and are, therefore, ground-tissue elements (Pl. XXVII, fig. 1), whereas all those which lie within this sheath belong to the vascular tissue. In those plants which have no bundle sheath the bast fibres which are adjacent to the bundles must be regarded as belonging to them.

The TERMINATION *of the* NERVES *in the* VESTIBULE *and* SEMICIRCULAR CANALS *of* MAMMALS. Read before the British Association, Glasgow, September, 1876, by URBAN PRITCHARD, M.D., F.R.C.S., Aural Surgeon to King's College Hospital, Lecturer on Animal Physiology, King's College, London. (With Plate XXVIII.)

IN this paper I propose giving the results of my investigations into the structure of the nerve-epithelium, as it is called, which contains the terminal distribution of the acoustic nerves in the vestibule and semicircular canals.

Up to the present this structure has been chiefly studied in the cold-blooded vertebrates and birds; and the various observers (including Max Schultze, Kölliker, Odenius, Hasse, Rüdinger, Ebner, and Meyer), although they agree as regards certain general points, yet appear to differ considerably respecting many important questions.

Having been fortunate enough to make some very successful preparations of this nerve-epithelium in the adult and fœtal cat, which to my mind throw considerable light on the subject, I have ventured to lay before you the results of these observations.

The area of distribution of the acoustic nerve in the vestibule and semicircular canals is limited to five spots,—one situated respectively in each division of this part of the membranous labyrinth; namely, the Saccule, Utricle, and three ampullæ. In the latter the area of distribution is raised into a kind of ridge or crest, and for this reason was named by Max Schultze the Crista Acustica; whereas in the Saccule and Utricle it is merely an oval spot, and has received the name of the Macula Acustica from the same author.

In the region of these acoustic ridges and spots the membranous labyrinth is firmly adherent, by means of a layer of dense connective tissue, to the bony wall, not being separated by the epilymph as in other parts of the labyrinth, and the membrane itself is specially modified in structure.

Speaking generally this membrane consists of three parts; externally an irregular layer of loose connective tissue; next a dense layer of the same tissue, frequently called the Tunica Propria; and lastly a single layer of tesselated epithelial cells. At the acoustic spots the outer layer is lost, becoming blended with the periosteum, which in many animals (sheep, guinea pigs, &c.), here presents a layer of branched pigment cells, such as are found in the choroid coat of the eyeball. The tunica propria remains almost unchanged, but in the epithelial layer are to be found some very remarkable modifications.

The tesselated cells as they approach the acoustic spot gradually increase in thickness, and become first cuboid and then columnar, in which form they are found on the margin of the acoustic spot, where they are changed into nerve-epithelium. But before proceeding to the detailed account of this structure, I must mention that scattered somewhat irregularly amongst the columnar epithelium are found (according to Hasse, Odenius, and Meyer), peculiar pigmented fibro-granular cells with small summits and extending bases, which frequently meet, giving the appearance of a pigmented network when looking at the membrane from above; these cells appear to be similar to those found so abundantly in the tegumentum of the bird's cochlea.

The Nerve-Epithelium.

Max Schultze in 1858 described this strncture in the fishes as consisting of three epithelial forms. A deep layer of nuclei which comes next to the tunica propria, these he called the Basalzellen; a superficial layer of cylindrical cells, Cylinderzellen; and numerous nuclei between them, sur-

rounded by protoplasm and having prolongations upwards and downwards, the former passing between the cylindrical cells, and the latter between the nuclei of the lower layer, —these middle cells he termed Fadenzellen. He admitted that he was unable to trace the exact origin of the cilia which project from the epithelial layer.

Odenius, Kölliker, and Max Schultze himself, in his later researches, found similar structures in the higher animals; and although they differed somewhat in details, yet they came essentially to the same results.

Hasse, in his earlier researches on the bird, found himself unable to corroborate the statements of Max Schultze, and he described the nerve-epithelium as consisting of two alternating forms of almost cylindrical epithelial cells. The one bearing the cilium he very aptly terms the thorn cell, and the other the isolating cell.

The thorn cells he describes as being fusiform, with the thorn projecting from its upper part; the isolating cells as enlarged at their base, which contains nuclei and rest on the tunica propria, the upper part of the cells being narrow, and passing between the thorn cells so as to separate them.

Rüdinger, in his description of this structure in 'Stricker's Histology,' somewhat reverses Hasse's statement, and depict it as consisting of fusiform ciliated cells alternating with triangular ones, with their bases turned upwards instead of downwards. At the same time he states that he has been unable to discover the deep layer of nuclei described by Max Schultze.

Ebner, in endeavouring to reconcile the opposing statements of Max Schultze and Hasse, came to a conclusion which differed from each. He considers that this nerve epithelium consists of two parts—a superficial layer of cylindrical ciliated cells with a cuticular border above, and rounded off below, and layers of fusiform cells made up of a nucleus and two fine prolongations, the upper one passing between the cylindrical cells as far as the cuticular border. The nuclei of this second set of cells correspond to the deep layer of Max Schultze.

Lastly, Meyer, in his elaborate work on the membranous labyrinth, just published, arrives at very different results. He describes a deep layer of nuclei, as Max Schultze, and above this a layer of cylindrical cells, with cilia projecting from their upper free ends, and abruptly tapering to a nerve filament below.

My observations have led me to conclusions which, although they are essentially different from those of the

authors just alluded to, yet appear to me to reconcile, to a great degree, their various conflicting descriptions.

As this structure is admitted by all observers to be virtually the same in the vestibule and ampullæ, a description of the macula acustica of the saccule will suffice for all.

Figs. A, B, and C (Plate XXVIII) represent a transverse section of the macula at different points, and is drawn from a preparation of the labyrinth of a cat ten months old.

A glance at the drawing will suffice to show that this nerve epithelium does not present the same appearances throughout the whole macula. I therefore propose, first, to describe the type of this structure, and then its modifications.

A typical portion, such as may be seen midway between the centre and circumference of the spot, consists of a row of alternating elongated cells, bordered by a distinct cuticular membrane. Below these is a nuclear layer, such as has been described by all recent observers with the exception of Rüdinger; however, connections may be clearly traced between the upper elongated cells and this deep layer of nuclei; so that the cellular elements of this tissue may be described as consisting of two alternating forms of elongated cells, each having an upper and a lower nucleus.

The first I call the thorn-cells, on account of the shape of their cilium, and the second the bristle-cells, for a similar reason.

The thorn-cells have a fusiform body, containing an oval nucleus; from this body passes upwards a single cilium, which gradually tapers to a point; this cilium or thorn passes through an opening in the cuticular membrane, but appears to be totally unconnected with it. The lower tapering extremity of the body passing downwards again expands and encloses a second oval nucleus.

The bristle-cells have a triangular body containing an oval nucleus; the base of this is intimately connected with the cuticular membrane, and from this base passes upwards a single narrow cilium or bristle, having parallel and not tapering borders, thus resembling in shape, though much larger, those found on the ciliated cells of Corti in the mammalian cochlea. The apex of this triangular body is prolonged downwards, and has a second enlargement containing a second oval nucleus; this is usually situated at a different level to that of the corresponding enlargement of the thorn cell, and thus the alternating cells appear to dovetail, as it were.

The cuticular membrane is a very thick, well-marked membrane, keeping the cellular elements in their place, and

perforated for the passage of the cilia. I am very much surprised that none of the previous investigators should have described this membrane; it is true that Ebner and Meyer hint at something of the kind, but the other authors appear to ignore it entirely. I can only account for this by the fact that in fœtal preparations this membrane cannot be made out.

This cuticular membrane is undoubtedly analagous to the reticulate membrane of the organ of Corti, and I therefore propose calling it by the same name—membrana reticularis.

Having described the type of the nerve-epithelium, I now pass on to its modifications. As there is a general increase in the thickness of the macula from circumference to centre, so the cells and their various parts elongate; the cilia, which are short and stumpy at the edge, become very much longer and comparatively finer at the centre of the acoustic spot.

Round the border of the spot the cells pass by insensible gradations into the columnar form of the epithelial cells which surround it. In this change the double nature of the cellular elements is very apparent.

Towards the centre of the acoustic spot the upper nucleus and surrounding protoplasm of the bristle-cells gradually diminish, and then are lost altogether, the cell being represented by a trabecula from the membrana reticularis, tapering downwards towards its second or lower nucleus. The bristle-like cilium remains after the upper protoplasmic mass has disappeared, but eventually this also is lost. Curiously enough, as this atrophy of the cell is proceeding, the bristle-like cilium appears to go out of its course to approach nearer and nearer to its neighbouring thorn, so that towards the centre of the acoustic spot the two cilia are seen lying close together, and almost appear to proceed from one and the same cell.

I think this description bears out what I just stated, that my observations tend to reconcile the opposing views of previous writers.

Naturally, the lower layer of nuclei has been recognised by all except Rüdinger, who seems to have been unfortunate on this point.

The isolating cells of Hasse and triangular ones of Rüdinger are represented by my bristle-cells, no one having previously observed their cilia, or, indeed, that there were were two forms of cilium. Again, these cells, where they lose their upper nucleus, correspond to the filiform cells of Max Schultze and Ebner.

Rüdinger's description is very like what may be seen in

fœtal preparations, where the cells retain much of their original epithelial form.

My thorn cells correspond to the cylindrical or fusiform ciliated cells recognised by all.

The Nerves of the Maculæ and Cristæ.

Having thus fully described the nerve epithelium, I now pass on to the nerves themselves, and will afterwards briefly describe the otolith mass covering the acoustic spots.

The vestibular or posterior division of the portio mollis separates into five branches, one for each of the areas of distribution. In the trunk of this nerve and its branches, but especially in the former, are found fusiform bipolar ganglion cells, corresponding to those of the ganglion spirale of the cochlea, but much larger, those of the latter averaging $\frac{1}{1000}$ inch in length, whilst the vestibular cells average $\frac{1}{600}$ inch.

These five branches pass through the bone to their respective destinations, and the fibres arriving at the membrana propria lose their white substance, entering the nerve-epithelium without it. After passing this point there is considerable difficulty in tracing the nerve filaments, but the later observers are all agreed, as no doubt is the case, that they found a plexus around the deep layer of nuclei, and that some of the filaments may be traced directly or indirectly into the ciliated cells. Meyer describes and figures minute nerve filaments forming a plexus and passing up between the cylindrical cells; the existence of these has not as yet been confirmed by my observations.

Rüdinger states that he has traced the nerve filament through the centre of the ciliated cell and its nucleus right to the cilium itself; I have sometimes fancied that I could also do this, and certainly there is a central darker portion to be seen in the thorn-shaped cilia.

The Otolith Mass.

Covering the acoustic spot is a soft mass of cuticular tissue, into which the cilia project to a certain distance. This mass has an irregular reticulate appearance in preparations, but is evidently of a cuticular nature and analogous to the membrana tectoria of the cochlea, with which it is continuous in the bird according to Hasse. The otoliths are fixed by this mass, being chiefly contained in its outer portion.

In conclusion, I would observe that, although I am confident that my explanation of this structure is the correct

one, and, further, that I can satisfactorily demonstrate most that I have put forward, yet further research is needed to render the matter more certain, and especially to clear up the question of the ultimate termination of the nerve filaments.

The following is the list of authors' works referred to; and to those who may be desirous of entering more fully into the history of the subject I would specially recommend the work of Paul Meyer on the 'Labyrinthe Membraneux,' just published.

Max Schultze.—'Ueber die Endigungsweise des Hörnerven im Labyrinth,' 1858. J. Müller, 'Archiv für Anat. u. Phys.'
Kölliker.—'Handbuch der Gewebelehre des Menchen,' 1867.
M. V. Odenius.—'Ueber das Epithel der Maculæ Acusticæ beim Menschen,' 1867. 'Archiv für Mikros. Anat.'
C. Hasse.—"Der Bogenapparat der Vogel," Siebold u. Kölliker, 'Zeitschr. für Wissensch. Zoologie,' Bd. xvii, p. 381.
C. Hasse.—'Das Gehörorgan der Fische.' Idem, p. 454.
Rüdinger.—'Stricker's Histology,' vol. iii.
Ebner.—"Das Nerven-Epithel der Cristæ Acusticæ," in 'Schriften des Med. Natur. Wissensch. Vereins zu Innsbruck,' 3 Jahrg., 1872.
Paul Meyer.—'Etudes Histologiques sur le Labyrinthe Membraneux,' 1876.

On some Foraminifera *from the* Loo Choo Islands. By Henry B. Brady, F.R.S.[1]

A pressed and mounted specimen of a small alga, labelled "*Laurencia paniculata*, Loo Choo Islands," was recently sent to me by Dr. E. Perceval Wright, with the suggestion that some Foraminifera which had been entangled in its meshes might be worth examination, and that at any rate it would be interesting to know what particular species had lived amongst its miniature branches. As the sea-weed itself was of some value, two or three inches were taken, and the portion as separated yielded examples of the following species of Foraminifera, some of them in sufficient number to supply two or three good mountings.

Hauerina compressa, d'Orbigny.
Quinqueloculina subrotunda, Montagu.
" *bicornis*, Walker and Jacob.
" *ornatissima*, Karrer.
Peneroplis pertusus, Batsch.

[1] From 'Proceedings Royal Irish Academy,' vol. ii, ser. 2, 'Science.' Read May 8th, 1876, published July, 1876.

Vertebralina striata, d'Orbigny.
Orbitolites complanata, Lamarck.
Discorbina rosacea, d'Orbigny.
„ *globularis*, d'Orbigny.
Planorbulina Mediterranensis, d'Orbigny.
Calcarina Spengleri, Gmelin.
„ *calcar*, d'Orbigny.
„ *hispida*, spec. nov.
Tinoporus baculatus, Montfort.
Cymbalopora Poeyi, d'Orbigny.
Heterostegina depressa, d'Orbigny.

These form altogether a considerable list, considering that the entire weight of sea-weed, shells, and extraneous adherent matter could not be more than fifteen or twenty grains. One or two forms were represented by a single specimen only, but no species had been retained of which a good characteristic example, large or small, were not present; doubtful forms were rejected, or the list might have been considerably extended. The most abundant species of Calcarina was the pretty hispid modification figured by Dr. Carpenter (Introd. Foram., Pl. xiv, fig. 6), but not hitherto described or named as far as I know. I propose to call this *C. hispida*, and its characters will stand as follow. I have met with larger specimens in Australasian sands, but have never seen any so beautifully perfect.

Calcarina hispida, *spec. nov.*—Test free, unequally biconvex, rotalian; margin, thin, lobulate or rowelled; segments numerous, slightly inflated; peripheral borders thin, rounded, angular, or produced sufficiently to form radiating spurs. Surface covered with adpressed spiny processes, obscuring the sutures, except those of the later chambers. Diamet. $\frac{1}{20}$ inch (1·3 mm.) or more (*vide* Carpenter, *op. cit.*, Pl. xiv, fig. 6). The characters are, indeed, very much those of *Calcarina calcar*, excepting for the superficial spiny armature.

Quinqueloculina ornatissima (Karrer, Sitzungsb. k. Akad. Wiss. Wien, 1868, vol. lviii, p. 151, pl. 3, fig. 2) deserves a passing notice. It is an interesting, highly ornate form with transverse crenulations crossed by longitudinal striæ, and though I had previously found it in some Polynesian sands, it has not been recorded as a recent species. Dr. Karrer's specimens were from the Miocene of the Banat, in Austria. Only a single example was found in this Loo Choo gathering, and that is slightly broken.

At the time I received the sea-weed from Dr. Wright I was endeavouring to summarise what was known of the

parasitic types of Foraminifera in connection with my work upon the Rhizopod fauna of the Carboniferous rocks, and I had arrived at the conclusion that adherent growth, at one period of life or another, was a much more common and more significant character in this group of organisms than has hitherto been supposed. It was therefore of interest to ascertain not only what species of Foraminifera were present, but how many of them, if any, were really parasitic, and not simply entangled in the meshes of the weed amongst which they had lived, or adherent by the mucilaginous matter coating the surface. The piece of the alga which had been separated, consisting chiefly of the root and the commencement of the larger branches, was therefore put into warm water and allowed to macerate for twenty-four hours, by which time it had swollen to its original size. Repeated sharp agitation during the maceration served to liberate most of the Foraminifera. It was then cut into little pieces, and the filaments of a conferva with which it had been associated in growth were carefully removed, the pieces were put into a sieve and washed under a strong stream of warm water from a tap, using every means even to the extent of some violence to dislodge anything that had not some connection with the surface of the plant beyond mere chance adhesion. The specimens that remained were comparatively few in number, and pertained to a limited range of species, but for the most part they had evidently lived in the parasitic condition in which they were found. They were chiefly the young of *Orbitolites complanata* and *Cymbalopora Poeyi*, with small examples of *Planorbulina Mediterranensis*. The last-named needs no comment, as it is an essentially parasitic species, but I am not aware that either *Orbitolites* or *Cymbalopora* has ever before been noticed in this condition. The little specimen of *Cymbalopora* might have passed for the fry of one of the other Rotalian genera, but for the presence of larger specimens of the same species.

REVIEW.

Epicrisis Systematis Floridearum. Auctore JACOBO GEORGIO AGARDH. Lipsiæ: apud T. O. Weigel, 1876.

AFTER an interval of thirteen years Professor Agardh has published, under the above title, the third volume of 'SPECIES, GENERA, ET ORDINES ALGARUM.' The first volume of the work, it will be remembered, contained the Melanospermeæ, the second, which was divided into three parts, contained the Florideæ. Of these parts, the first was published in 1851, the second in 1852, and the third part, which treated of the RHODOMELEÆ only, in 1863.

Since the issue of the earlier parts immense numbers of Algæ, including a great many new to science, have been brought from all parts of the world, and have been submitted to scientific and accurate examination by algologists. The result of this examination has led to an acknowledgment that the former system of classification, based upon the study of insufficient materials, which were frequently misunderstood, was defective, and that a new classification was necessary. With this view the present volume has been prepared.

As it is now understood that the determination of Algæ depends not so much upon outward form as upon microscopical examination of the internal structure and fruit, no apology is necessary for giving an account of Professor Agardh's work in this Journal.

The preface first claims attention, because in this the professor refers to some points important to algologists.

He notices first the mimicry of forms which is a frequent feature in Algæ as in other branches of natural history. Dr. Harvey, in his beautiful work, the 'Phycologia Australasica,' had before observed this, and had called attention to the resemblance in outward appearance between *Delisea pulchra*, *Phacelocarpus Labillardieri*, and *Ptilota striata*. The resemblance, however, is only external, the internal structure and the fructification being in each essentially different. The instances cited by Professor Agardh are still more striking, because in them not only the outward form but the

structure of the plants are exactly similar, while the fructification is entirely different. As instances he cites some forms of Callithamnion and of Wrangelia, of which it is impossible to determine the genus without microscopic examination of the fruit—and the fruit, alas! is too often absent in specimens submitted for examination. Hence Professor Agardh draws the conclusion that none of the Florideæ can be considered to be correctly placed in the system, whose internal structure and fruit are not known to us by numerous examples; nor can any dependence be placed upon descriptions or figures of sterile forms or analyses of fruit and structure (often either erroneous or defective), of which only a few examples are known. Acting on this principle, he tells us that in this volume, as in the preceding, he has inserted no species in the text which he has not himself examined. Species which he has not seen are either inserted in notes or placed at the end of each genus as "species enquirendæ." A few species, easily understood, have been, however, admitted on the faith of well-executed figures.

"If," says Professor Agardh, "resting on the fact before us (the imitation or repetition of forms), we go on to conclusions, is it not obvious that the reports we have heard about the arrival of our Algæ on distant shores are by no means to be believed, unless the species of different localities have been subjected to accurate examination, which, although algologists had the Algæ before them, they have generally omitted to make? When we have had an opportunity of comparing specimens from different places, we have shown that these reports are false. With regard to algologists, as to early travellers who have made similar assertions about the higher plants, it has been their error that they regarded nature, not only as not copious, but as poor and scanty."

In the 'Phycologia Australasica' sixty species are enumerated as common to the Australian colonies and to this country. On looking through the work before us it will be seen how very few of these are found to be exactly identical; many are well-marked varieties, and not a few are essentially different in generic characters. The same remark applies to Algæ from other countries said to be identical with some of those found on our coasts.

Professor Agardh, than whom, from his long and wide experience, no one is more competent to form an opinion on the subject, next takes occasion to express his belief in the immutability of species.

With regard to the determination of the genera of the FLORIDEÆ the professor states that the characters of most

importance are the structure of the cystocarp and the formation of the nucleus, and from long experience he expresses his conviction that where these characters do not agree there is no affinity. He had previously carried out this principle in his arrangement of the *Rhodomeleæ* (Vol. II, Part III), whose forms from lowest to highest form a continuous and natural ascending series. Besides the cystocarps, the various forms of sphæosporic fruit have been made use of in the classification wherever it was possible.

As to the "Trichogyne," of which so much has recently been written, it will be more satisfactory to the reader for the author to express his own views in his own language. He says (p. v)—

"Me in hoc volumine, quod potissimum dispositionem et descriptionem formarum spectat, nihil dixisse de organis, quæ maximi momenti considerarunt alii—de trichogynico ad paratu et corpusculis antheridiorum, horumque organorum functionibus—ignoscant precor. Quicumque oculo paulisper critico observationes et conclusiones inde deductas repetat, vix non videat observationes hucusque factas et ulterius repetendas et corroborandas esse. Mihi ipsi, adparatum trichogynicum in pluribus quærenti, an unquam verum videre contigerit, nescio. In iis cytocarpiis, quæ transformatione ramuli oriri videntur, ramuli transformati apicem fere sub eâ formâ nunc in fructu juvenili persistentem vidi, quam trichophorico adparatui tribuant. Confiteor me his locis in hoc adparatu nihil suspicasse, nisi apicem ramuli, ob evolutionem fructus hebetatum et sensim obsoletum. In Dudresnaja totam evolutionem nuclei a filo generante observare credidi; hoc loco evidentissimum mihi adparuit filum elongatum, quod nucleam superat, nihil esse nisi apicem sterilem decoloratum fili nucleum generantis. Quod attinet eos minutissimos globulos, qui corpuscula antheridiorum, trichogynis adfixa sisterent, patet eadem quoad originem et naturam omnino dubia esse. Non tantum sæpissime evenit ut in guttâ aquæ corpuscula diversissimæ naturæ circumvehantur, sed etiam practicis algologis bene constat sporas algarum et particulas diversæ naturæ Algis sæpe adfixas obvenire. Quomodo igitur probetur corpuscula illa, quæ trichogynis adfixa viderunt, fuisse corpuscula ex antheridiis provenientia, et ex antheridiis ejus speciei, quæ iisdem fœcundaretur? Si constaret antheridiorum corpuscula esse agilia, facilius quidem intelligeretur corpuscula antheridiorum trichogynicum adparatum potuisse attingere; sed corpuscula omni mobilitate destituta contendit ipse Thuret. Si denique qui ad totam rem illustrandam plurima contulerunt de

maxime essentialibus dissentiant, patet ut crediderim totam rem novis et iteratis observationibus antea esse confirmandam, quam ipsis organis descripsis quandam vim systematicam attribuamus."

Having thus referred to the principal points noticed in the preface, it remains to say a few words respecting the work to which it forms the introduction. The volume, which consists of upwards of 700 pages 8vo, contains descriptions of all the orders and genera of the Florideæ (with the exception of the *Corallineæ* and the *Rhodomeleæ*) and of all the new species comprised therein. The species which were correctly described in the preceding parts of Volume II have not been repeated, but are merely referred to. Where new characters were perceived and more precise definitions were necessary, they will be found in the present volume. It will be seen, therefore, that the work is strictly supplementary.

The arrangement, although based on the system of classification followed in the former work, is almost entirely new. There are twenty-two orders, arranged under six series and 173 genera; namely, 169 in the body of the work and 4 in the addenda. The list of new species is very extensive, large collections of Algæ having been made from the coastsof all the Australian colonies, New Zealand, and the Pacific coasts of America. Of late years the Swedish expeditions have brought home large collections of Algæ in excellent preservation; among these may be specially noticed those of Dr. Berggren in the Arctic seas, on the coasts of New Zealand, and on the Pacific shores of America. One cannot help contrasting these rich harvests of Algæ with the meagre lists furnished by Dr. Dickie, and published in the 'Journal of the Linnean Society,' of the Algæ collected by "the Challenger expedition" during its three years' cruise. The work just published by Mr. Moseley 'On True Corals' proves that zoological investigations are more to his taste than collecting and preserving Algæ.

With regard to the Melanosperms, of which the first volume of 'SPECIES ALGARUM' treats, it may be mentioned that Professor Agardh has recently published a treatise on the LAMINARIEÆ and FUCACEÆ of Greenland,[1] and has revised and rearranged the genera CYSTOPHORA and ZONARIA, and so much of the genus SARGASSUM as includes the tribes *Pterocaulon*, *Phyllotricha*, *Schizophylla*, and *Heterophylla*—so far as they relate to Australian species—several new species being added. With the latter is published a monograph of

[1] "Bidrag till Kännedomen af Grönlands Laminarieer och Fucaceer," 'K. Vet. Akad.,' Sept. 27, 1871, Stockholm.

the genus CAULERPA,[1] the only portion of the CHLOROSPERMS that has yet appeared. It is to be hoped, but perhaps scarcely to be expected in the present state of our knowledge of this large class of Algæ, that another volume containing the whole of the CHLOROSPERMS will soon follow the present. Such a work is known to have been long in hand, but Professor Agardh is not one who hurries in the execution of his work, but who seeks above all things to do that work accurately and thoroughly. In the present state of uncertainty which prevails regarding the affinity between the Algæ and Lichens, wise men are cautious in expressing opinions which may or may not prove to be supported by facts.

MARY P. MERRIFIELD.

[1] "Till Algernes Systematik. Nya Bidrag af J. G. Agardh," 'Lunds Univ. Arsskr,' tom. ix, 1872.

NOTES AND MEMORANDA.

Dr. Klein's supposed Mycelial Growth in Sheep-pox.—The remarkable appearances detected by Dr. Klein in the tissues of sheep suffering from variola ovina, which were considered by many eminent naturalists after inspection of his preparations to be parasitic organisms similar to the mycelium of a fungus, have been proved to be artificially produced coagula of albuminous matter. Dr. Klein's report to the medical officer of the Privy Council and his drawings were reprinted in this Journal last year. At the last meeting of the Royal Society, in June, a paper was read by Dr. Creighton, lately engaged in the Brown Institute, in researches on the pathology of cancer, in which that gentleman showed that appearances similar to those obtained by Dr. Klein were obtained abundantly in preparations of healthy tissue treated when perfectly fresh with hardening reagents. Dr. Creighton's preparations consisted chiefly of sections of the mammary gland. In a note read at the same time Dr. Klein accepted the explanation of the mycelium-like appearances given by Dr. Creighton. Though the supposed proof of the parasitic nature of variola has thus been swept away, it must not be supposed that there was not large justification for regarding the coagula as specific organisms. The filamentous branched and knotted appearance which they presented was strangely like that of an organic growth. Their minuter structure, whilst differing from that of an ordinary fungus-mycelium in showing no differentiation of a cell-wall, agreed with such in the presence of vacuole-like spaces and in the excessively fine granular character of the supposed protoplasmic substance. In many cases the branching filaments met together and formed a network, which is unlike any growth exhibited by an ordinary fungus-mycelium, but suggestive of a naked protoplasmic mycelium, similar to that of the Myxomycetes. Whilst we have, then, lost, or rather not gained, an advance in our knowledge of the contagium of variola, we have gained a very important lesson in the necessity for being on our guard as to such

artificial results due to our methods of manipulation as are these mycelioid coagula. Already in another branch of histological inquiry observers have been on their guard against the deceptive appearances of membranes and fibres produced by the coagulation of albuminous fluids when acted upon by the hardening reagents used in the laboratory. One of the most careful and accomplished of the younger German histologists, Dr. Flemming, of Kiel, makes the following remarks in a memoir on the "Anatomy and Physiology of Connective Tissue" ('Archiv für Mikrosk. Anatomie,' 1876, vol. xii, p. 396):—"I have worked for some time with this combination (osmic acid and anilin), and have found, as is well known, no doubt, to others, that one must not accept as veritable structure all that it brings into view. Notoriously, osmic acid, like other hardening reagents, produces coagulation of the albuminous fluids of the body, such as blood, serum, and lymph, and the coagula become deeply tinted with the anilin dye. In fact this kind of coagulum can be obtained without previous application of osmic acid by the action of the simple solutions of the anilin pigments, most abundantly, as one might expect, by the use of alcoholic solutions, but also by the use of aqueous solutions. I would never undertake to say that membranous-looking things, such as this treatment has yielded me, with loose connective tissue, are really membranous and not coagula." Professor Flemming observes that one can most satisfactorily convince oneself of the occurrence of these albuminous coagula in blood- and lymph-vessels by the study of animals which have not been killed by bleeding (a case which applies very well to the excised portions of tissue studied by Dr. Klein), and, perhaps, best of all by the study of embryos; the parietal cavities and the vessels of the latter are almost always, after treatment with osmic acid, filled with coagulated masses which take up the staining.

A knowledge of the simulation by these coagula of definite organic form so closely as to mislead experienced mycologists is the not unimportant result of the researches of Dr. Klein and Dr. Creighton.

PROCEEDINGS OF SOCIETIES.

Dublin Microscopical Club.

20*th April*, 1876.

On a hitherto unnoticed problematic and presumably parasitic unicellular growth in the cavity of the Cells of Blodgettia, Harvey.—Professor E. Perceval Wright drew attention to another presumably parasitic growth in the cavity of the cells of "Blodgettia" (Harvey), consisting of colourless thick-walled cells. Occurring more or less in rows, and sometimes giving the appearance as if connected by linear stipites, these were wholly distinct from and far smaller than the presumed spores of Harvey's Blodgettia. If these latter really belong not thereto, but are a foreign parasite (the question raised by Dr. Wright at last meeting of the Club), then one and the same "Cladophora" would seemingly be the host of two distinct parasitic growths. If, on the other hand, fresh examples should hereafter demonstrate the tenability of Harvey's genus, then at least it would seem to harbour the growth to which Dr. Wright to-night drew attention. The crystals described and figured by Harvey were also present.

Diatomaceous forms from Rockett River, Sierra Leone, were exhibited by Rev. E. O'Meara, who directed attention therein to a form of Coscinodiscus strongly resembling *C. perforatus*, but differing in having the hyaline areoles around the hyaline centre cuneate instead of round.

Structures of the leaves of Pinus (*Pseudotsuga*) *Douglasii* (= *amabilis*, Parlatore).—Professor W. R. McNab exhibited sections of the leaf of the Pine collected in New Mexico by Fendler, and numbered "829." The specimen was from the Herbarium of Trinity College, Dublin, and had been placed at his disposal for examination by Professor Perceval Wright. Parlatore refers this plant to his *amabilis*, but Dr. McNab considered it to be *Pinus* (Pseudotsuga) *Douglasii*. In the parenchyma of the leaf were well-developed idioblasts, sometimes $\frac{1}{50}''$ across. The idioblasts were stellate cells with many arms, and having the walls very much thickened. The enlarged thickened cells are chiefly arranged on each side of the fibro-vascular bundle forming the midrib, but were not limited to that locality. Dr. McNab has met with similar cells in all the wild specimens of *Pinus Douglasii* he had examined, but had not been able to detect them in cultivated plants.

Section of Quartz-rock from Shankill, Co. Dublin, exhibited.—

Mr. Moss exhibited a section which he had made of the quartz-rock of Shankill, and pointed out that this rock, which is really a sandstone, consisted of rounded grains of quartz, held together by a siliceous cement, corresponding to the quartz-fragments in optical properties when examined by polarized light. The specimen contained numerous opaque specks of irregular form, apparently ferruginous, as a chemical analysis of this rock had revealed the presence of a notable quantity of iron.

New species of Closterium, exhibited.—Mr. Archer exhibited a seemingly undescribed species of Closterium. Small, comparatively stout, curvature slight, inflated at the middle, gradually tapering, though still thick towards the apices, which are blunt, rounded; membrane smooth and colourless; endochrome a single longitudinal band, not plicated; each loculus, containing usually but a single moving granule, forming a rounded cavity in the plasma, at some distance from the apex. All the other smooth species are greatly more curved and the apices more attenuated and by no means so broadly rounded as in this new form, which might stand as *Closterium monotœnium*.

11*th May*, 1876.

Ectocarpus Farlowi, Thuret, *and a very problematic algal production collected by the late Professor Harvey, exhibited.*—Professor E. P. Wright exhibited a pretty species of Ectocarpus, named by the late M. Thuret after Mr. Farlow, assistant to Professor Asa Gray; the specimens exhibited had been collected by Mr. Farlow at Eastport, October, 1875. Dr. Wright also exhibited a very paradoxical algal production, collected by the late Professor Harvey on mangrove stems, at Vavau, Friendly Islands. But a few specimens were obtained, and the only record made—beyond the date and locality—was 99. "? ? Alga ? quam maxime paradoxa!" The form was certainly a paradoxical one—a mass seemingly made up of linear, truncate green cells, forming a confused congeries; without fresh specimens preserved in fluid it would be impossible to do more than suggest that it had affinities to such genera as Hydrodictyon or Pandorina, but then something of its reproduction should be known ere it would be safe to include it in that section of Zygosporeæ with conjugation of the "swarm-cells."

Structure of leaves of Abies Veitchii, Lindl., *and of the species so called in gardens, which are really distinct.*—Dr. McNab exhibited transverse sections of the leaves of *Abies Veitchii*, Lindl., and of the species *called Abies Veitchii* which he had received from Messrs. Veitch, of Chelsea, and other nurseries and gardens. *Abies Veitchii*, Lindl., has the resin-canals in the parenchyma of the leaf, and has hardly any hypoderma; the leaf is blunt, and in many respects like that of *Abies Pectinata*, D.C. The *Abies Veitchii* of gardens is a distinct species, with the resin-canals close to the epidermis of the under side of the leaf and with the hypoderm exceedingly well developed. The leaf is more like that of *Abies Cephalonica*, with a sharp, generally bifid apex, and not like *A.*

pectinata. Dr. McNab proposed to call this form provisionally *Abies Harryana*, after Mr. Harry J. Veitch, the head of the firm of Veitch and Sons, Chelsea.

Film of vitreous Selenium, exhibited.—Mr. R. J. Moss exhibited a transparent film of vitreous Selenium about $\frac{1}{1200}$″ in thickness, and containing a dendritic mass of the same element in the crystalline form. He referred to the allotropism of Selenium, and stated that this early stage of the transition from the vitreous to the crystalline state had not hitherto been observed.

New Cosmarium sp. exhibited.—Mr. Crowe showed a Cosmarium gathered by him in the greatest purity and abundance in a pool near Bangor, North Wales, which appeared to be undescribed. It is of rather large size, granules fine and scattered, semicells in figure resembling those of *Cosm. biretum*, but in end-view elliptic, the lateral margins with a very slight median prominence (not with a large rounded inflation at each side). Mr. Archer had forwarded examples to Mr. Roy, of Aberdeen, and to Herr Nordstedt, of Lund, with a view to gain the opinions of those observers.

Cosmarium pseudomargaritiferum, Reinsch, *ex herb. Reinsch, exhibited with its zygospore, and note thereon.*—Mr. Archer showed authentic examples of *Cosmarium pseudomargaritiferum*, Reinsch, showing the peculiar zygospore. This form, though smaller in dimensions, appeared the same as *Cosmarium Portianum*, Archer. Professor Reinsch describes the zygospore as *smooth*—certainly it is not spinous—but it is deeply scrobiculate, a character which that observer does not record. In this it seems to resemble no other zygospore save that of the otherwise dissimilar *Xanthidium armatum*, and hence, in the genus Cosmarium, it is (as yet) unique. The zygospore of *Cosmarium margaritiferum*, or better *Cosm. reniforme* (*Cosm. margaritiferum*, *β reniforme*, Ralfs) is much larger, very thick-walled and absolutely smooth (not scrobiculate), whilst that of *Cosm. margaritiferum proper* is large, thick-walled, and covered by thickened subhemispherical hyaline prominences (comparable to "bull's eyes" in glass); thus the three forms are distinct in every particular, and the suggested or implied close affinity to one or other of the other species in question conveyed by the name "*pseudomargaritiferum*" appears altogether unjustifiable.

Eurotium herbariorum from tan-bed exhibited.—Mr. Greenwood Pim exhibited specimens of a Fungus which had appeared in great abundance on a tan-bed in his garden, so numerous as to give quite a rusty tinge to the tan; further investigation showed they were immature conceptacles of *Eurotium herbariorum*, Lv.

8*th June*, 1876.

Diatomaceous forms, with a new species of Colletonema, from sub-fossil material forms along with remains of Megaceros.—Rev. Eugene O'Meara presented a slide containing Diatomaceous forms found in a subfossil material from Ballybetagh, under the stratum in which were embedded numerous remains of the extinct

"Irish elk." It contained nearly all the species of Epithemia incidental to the freshwater and in the greatest abundance. More sparingly were found some common species of Navicula, such as *N. major*, *N. nobilis*, *N. viridis*, &c.; *Campylodiscus costatus* also occurred. But the most remarkable form was one which Mr. O'Meara considered to belong in all probability to the genus Colletonema. The tubes, with the frustules included, were numerous, greatly broken, and ever appearing to be simple. The included frustules were very small, decidedly navicular, with very faint striæ. Mr. O'Meara proposed to designate this form as *Colletonema Hibernicum*.

Conjugated state of undescribed Cosmarium shown at preceding meeting of the Club.—Mr. Crowe showed the conjugated state of the Cosmarium brought forward by him at the last meeting of the Club, it having entered into that state very copiously in the house. The zygospore is large, thick-walled, and smooth, thus like that of *Cosmarium Broomei*, to which species, however, this shows no further resemblance. It was very interesting to meet with the zygospore so promptly in the discovery of the form itself, of which more on a future occasion.

Fruit of Anthoceros punctatus and preparation of the frond, exhibited.—Mr. Mackintosh brought forward a longitudinal section of the young fruit of *Anthoceros punctatus*, showing the columella running up the middle and the spores arranged on each side. He also showed a preparation of the growing edge of the frond, each of the cells of which had the characteristic single, large, irregularly stellate chlorophyll-body. The specimens were given to him by Professor E. P. Wright; they had appeared spontaneously in some peat in the orchid-house of the College Botanic Gardens, which had been brought from a bog near the "Sugar-Loaf" Mountain.

Cosmarium annulatum, Näg., *and three neighbouring new forms, exhibited.*—Mr. Archer exhibited three cylindrical forms of Cosmarium, one of which he thought to be identical with *Cosm. annulatum*, Nägeli, and a drawing of a fourth; three of the four now drawn attention to he believed to be new. Two were truncate (one of these being the *Cosmarium annulatum*), and two were rounded at the extremities. Mr. Archer would hereafter, he hoped, be able to prepare descriptions of these forms in full, and merely brought them forward now as a little series of kindred forms to show how closely such species could resemble and still be evidently mutually distinct.

Structure of leaves of Abies bifida and A. firma.—Dr. McNab exhibited transverse sections of the leaves of *Abies bifida* and *A. firma*. *A. bifida* is distinguished by having the resin-canals close to the epidermis of the under side of the leaf, and by having peculiar elongated thickened liber-like cells in the parenchyma of the mesophyll. *A. firma*, on the other hand, has the resin-canals in the parenchyma of the mesophyll, and has no thickened liber-like cells. The two forms are very distinct although given as synonymous by Parlatore.

A specimen of *A. brachyphyll-maxima*, Parlatore, proved to be the same as *A. firma*. All the specimens yet examined cultivated in Britain as *A. firma* are *A. bifida*.

Medical Microscopical Society.

An interesting soirée was given by this Society in the rooms of the Century Club, Pall Mall Place, on Friday, June 25th. The rooms were well filled, and numerous important pathological specimens, as well as new instruments, were exhibited. Among the former were a series of twenty typical specimens of tumours, by Messrs. Needham and Pritchard; alveolar sarcoma, by Mr. Godlee; tubercle of omentum, by Dr. Payne; absorption spectra of bile, &c., by Professor Ray Lankester; micro-photographs, by Mr. Fowke.

Among the instruments were a freezing microtome on a new principle, by Mr. R. C. Williams; microtomes on Rutherford's and Ranvier's principle, by Dr. Pritchard and Mr. Golding-Bird; a micro-photographic apparatus for taking photographs without a heliostat, by Mr. Giles; improved form of Mr. Stephenson's binocular microscope, by Mr. Bevington; a "differential" warm stage, by Mr. Golding-Bird; besides micro-spectroscopic apparatus, by Mr. Browning; and microscopes, by Beck, Pillischer, Verick, &c.

The specimens occupied the attention of members and visitors till a late hour.

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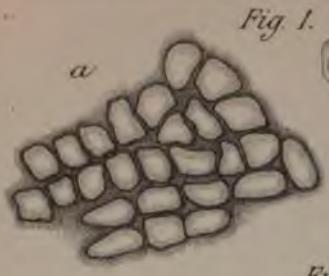

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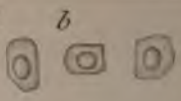

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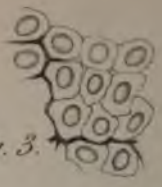

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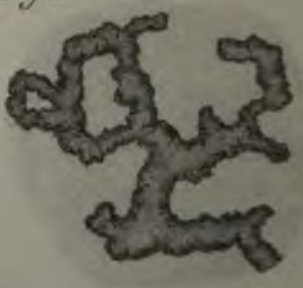

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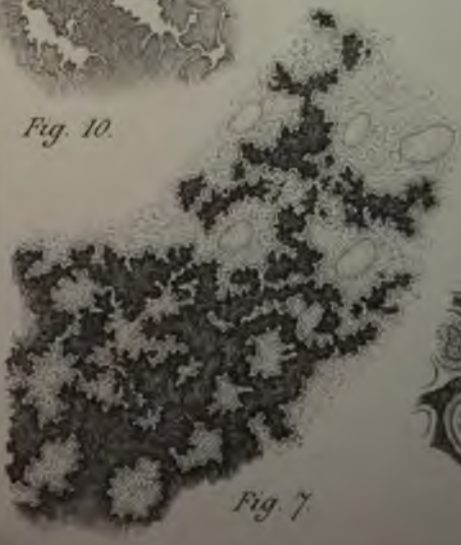

Fig. 7.

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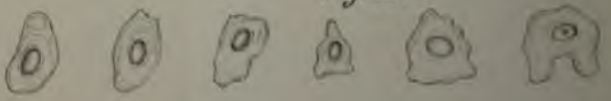

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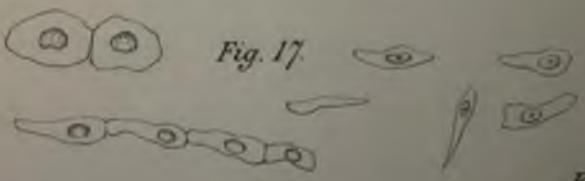

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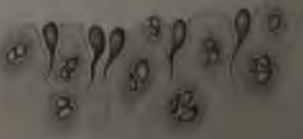


EXPLANATION OF PLATES I & II.

Illustrating Dr. Thin's Memoir on the Structure of Hyaline Cartilage.

(All the figures have been drawn as seen magnified by Hartnack's obj. 8, ocular 3; tube out.)

FIG. 1.—(*a*) Cells on the surface of the head of the femur of frog. (Nitrate of silver.) (*b*) Individual cells in which the outlines of the nucleus were distinct.

FIG. 2.—Part of a layer of cells isolated from the cartilage of the head of the frog's femur by solution of potash.

FIG. 3.—Cells lying on cartilage of head of frog's femur. (Solution of potash.) The cartilage-substance is not shown. The preparation showed convex and concave surfaces on which the cells were applied. An inter-epithelial substance is shown.

FIG. 4.—Part of a layer of cells from one of the tracheo-laryngeal cartilages of the frog. (Solution of potash.)

FIG. 5.—Part of a surface from the cartilage of the head of frog's femur covered with cells similar to those shown in the drawing. (Solution of potash.)

FIG. 6.—Transverse section through the condyle of a frog's femur. Nitrate of silver. (The whole of the epithelial surface seen is not drawn.)

FIG. 7.—Transverse section of cartilage from knee of a frog that died. (Nitrate of silver.)

FIG. 8.—Deposit of silver in the substance of a thick section from the head of a frog's femur which was rubbed over with lunar caustic.

FIG. 9.—Section through condyle of femur of a young rabbit, near the bone. Silver preparation. Deposit in centre of framework.

FIG. 10.—Section through condyle of femur of a young rabbit. Silver preparation. The white spaces are on the surface of the section.

FIG. 11.—Section through condyle of femur of one-day-old kitten. Silver preparation. Unstained surface continuous with several "spaces."

FIG. 12.—Tracheo-laryngeal cartilage of frog rubbed over with lunar caustic.

FIG. 13.—Section through condyle of femur of frog. Irritation of articular surfaces for seven days. Condyle was three days in ¼-per-cent. solution of osmic acid.

FIG. 14.—Section from condyle of femur of one-day-old kitten. Surface of cartilage rubbed over with lunar caustic.

FIG. 15.—Transverse section of cartilage of carpus of sheep sealed in aqueous humour.

FIG. 16.—Cells isolated in aqueous humour from sealed section of cartilage of carpus of sheep.

FIG. 17.—Cells seen in substance of sclerotic of frog sealed in aqueous humour.

FIG. 18.—"Giant cell" (disintegrated membranous substance) from section of cartilage of carpus of sheep after being seven months sealed in aqueous humour.

FIG. 19.—Isolated cylinder (= primary bundle of the author) from cartilage of head of frog's femur. In blood-serum.

FIG. 20.—Cells with processes seen in oblique edge of section of cartilage of carpus of sheep sealed in aqueous humour.

EXPLANATION OF PLATE III,

Illustrating Professor Lankester's Memoir on *Bacterium rubescens*.

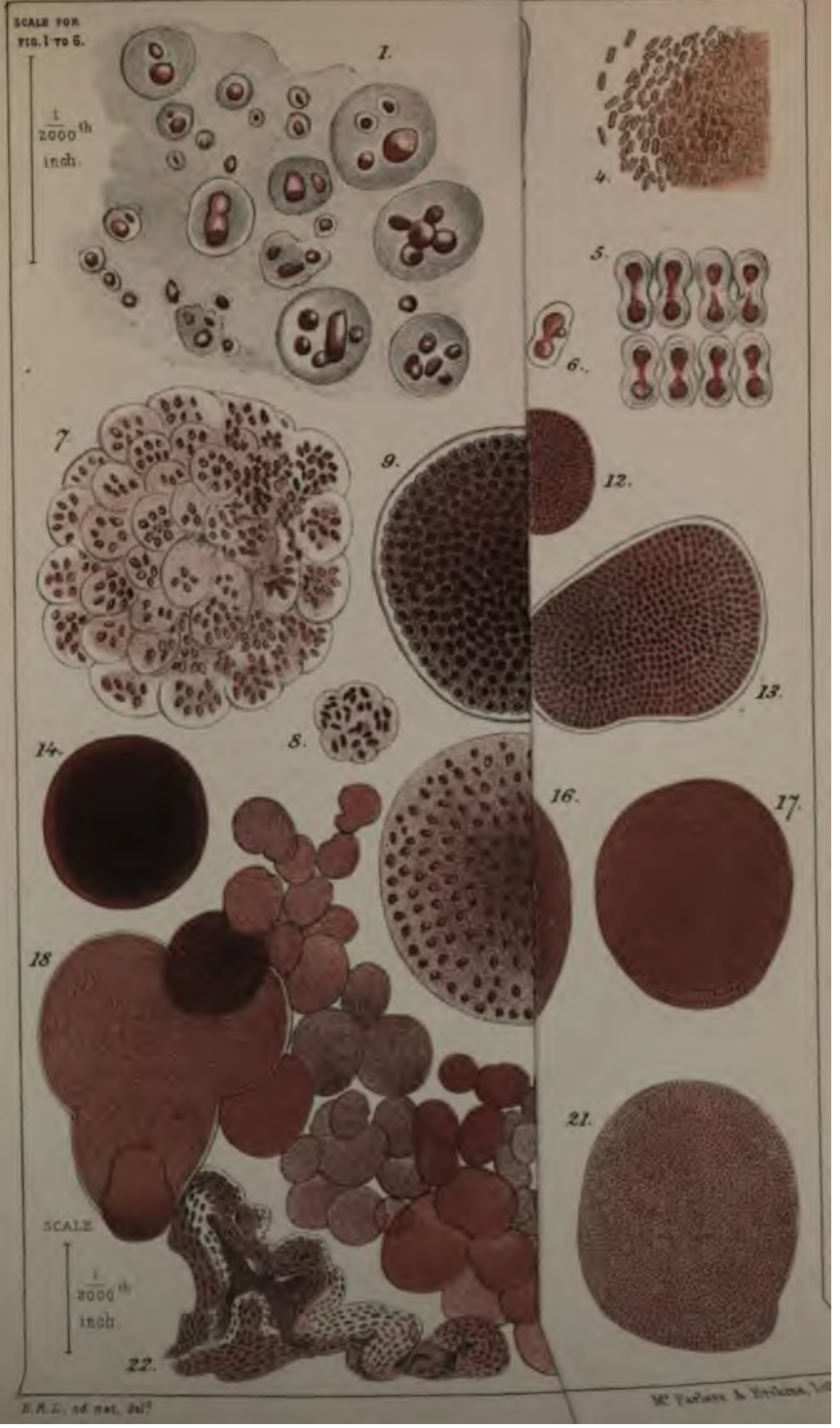
N.B.—The scale in the upper left-hand corner gives the amplification of figs. 1—6; the scale in the lower left-hand corner refers to the remaining figures.

FIGS.

1.—Multilocular glœogenous spherical plastids embedded in a continuous jelly-like matrix.
2.—Homogeneous biscuit-shaped plastids, freely swimming.
3.—Multilocular biscuit-shaped plastids, freely swimming. The more elongated specimens were motionless and are transition forms leading to the filamentous form of plastid.
4.—The maceration-breed of homogeneous biscuit-shaped plastids, comparable to *B. termo*. Some are in active movement.
5.—Rectilinear tesselate aggregate of unilocular biscuit-shaped plastids resembling *Merismopædia*. Similar sheets often attain six times the linear dimensions here given, and are composed of a proportionately larger number of rows of plastids.
6.—A plastid similar to those constituting the plate drawn in fig. 5, but possessing a small eccentric loculus. Compare fig. 22 of former memoir.

Figs. 7 to 23 (excepting figs. 10 and 22) represent phases in the development of the Macroplasts.

7.—Discoidal aggregate consisting of large glœogenous, spherical multilocular plastids.
8.—Detached plastid from a similar aggregate, showing its tendency to break up into smaller plastids.
9.—Macroplast with jelly-like border and closely compressed constituent plastids.
10.—Fragment of Clathrocystis-like tesselate aggregate, built up of unilocular biscuit-shaped plastids.
11.—Smallest-sized macroplasts.
12.—Small granular macroplast.
13.—Macroplast with coarser granulations (new free-formed plastids) and jelly-like border.
14.—Intensely coloured homogeneous macroplast.
15.—Loose condition of a macroplast. The free-formed plastids (or loculi) are now separated by abundant interposed jelly-like matrix.
16.—Homogeneous macroplast.
17.—Finely granular macroplast.
18.—Finely granular macroplast of irregular shape.
19.—A group of small macroplasts, drawn from an actual specimen.
20.—Homogeneous macroplasts of brownish tint.
21.—Finely granular macroplast.
22.—Fragment of a Clathrocystis-like tesselate aggregate, built up of unilocular biscuit-shaped plastids.
23.—Small macroplast of intense coloration.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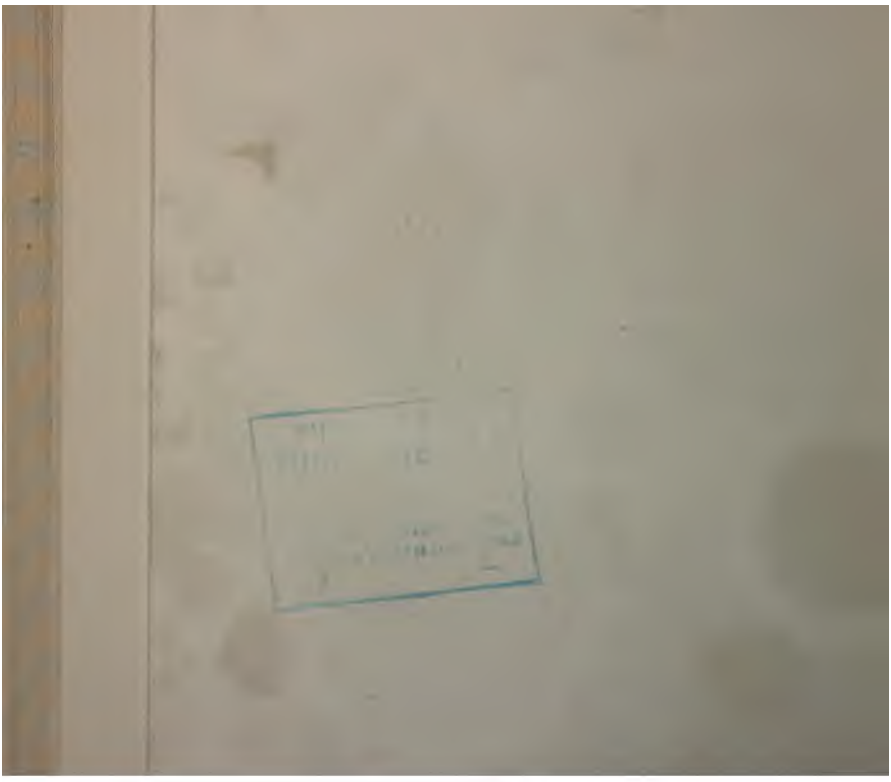

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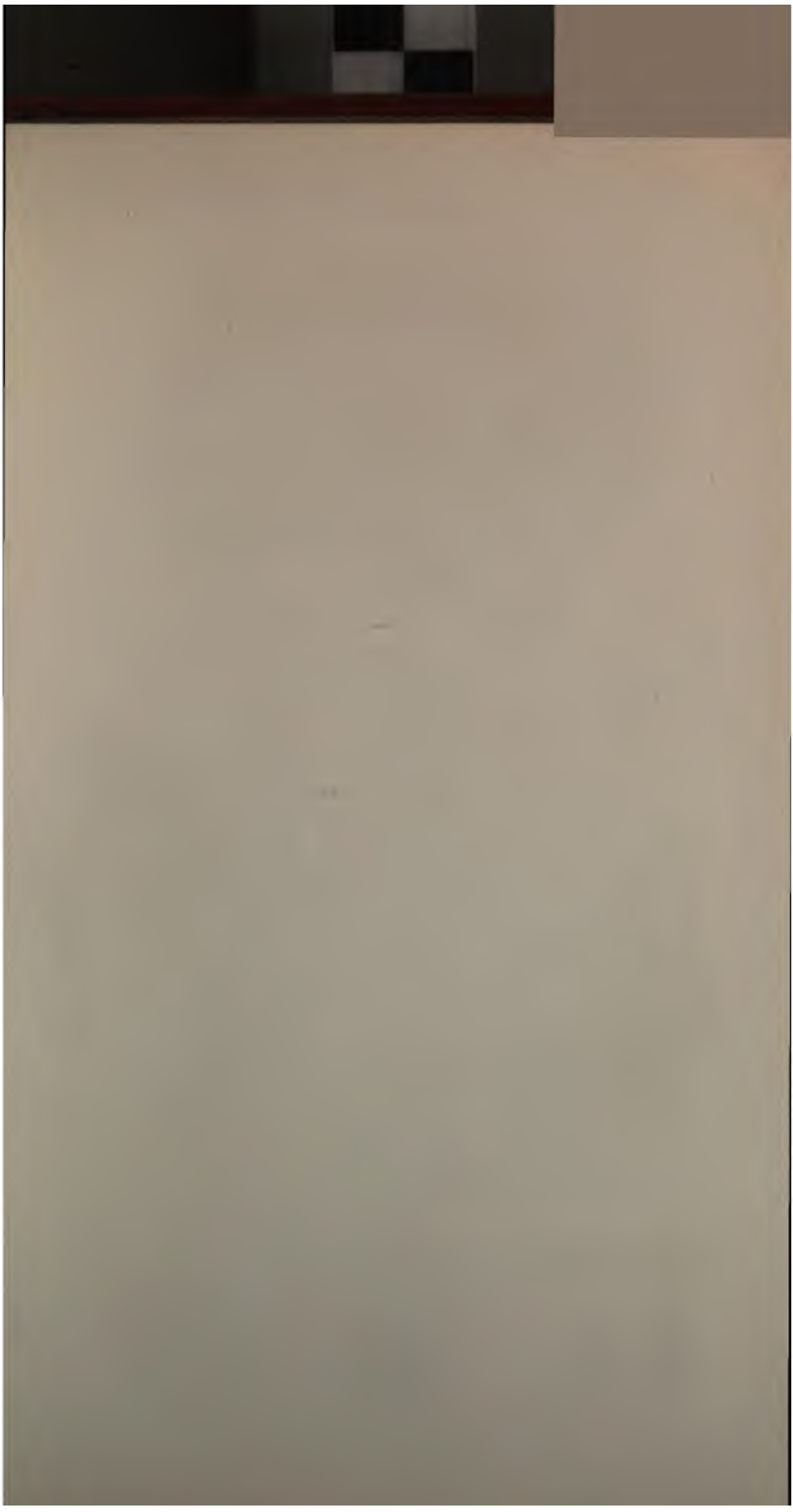

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

EXPLANATION OF PLATES IV & V.

Illustrating Mr. Tomes's paper on the Development of Teeth.

The lettering is the same in all the figures.

a. Oral epithelium.
b. Bone of the jaw.
c. Neck of the enamel organ, connecting it with the oral epithelium.
d. Formative pulp of the dentine = dentine papilla.
e. Enamel organ.
f. Stellate tissue of the enamel organ.
g. Cap of formed enamel.
t. Completed teeth, already in use.

FIG. 1.—From the lower jaw of a kitten, just born.

To the left of the larger tooth germ, which belongs to the milk tooth, is the smaller and younger germ of its permanent successor. The enamel germ of the permanent tooth-sac still retains its connection with the neck of the enamel organ of the deciduous tooth, whence it was originally derived.

FIG. 2.—From a green lizard.

A young tooth germ, consisting of an enamel organ with an excessively long neck, and a dentine germ.

FIG. 3.—From a green lizard (same specimen as No. 2), showing the relative positions of the older tooth and of the successional tooth germs.

FIG. 4.—Lower jaw of common English snake.

The successional tooth germs, four in number, are seen in transverse section, owing to the fact that the developing teeth lie recumbent and almost parallel to the long axis of the jaw.

FIG. 5.—Upper jaw of common frog.

To the inner side of the base of the tooth is that dipping inwards of epithelium (*c*) which is the first step towards the formation of a new enamel organ and new tooth germ.

PLATE V.

FIG. 6.—Transverse section of upper jaw of a newt.

To the right of the working tooth, which is tipped by a double point of enamel, are two younger tooth-sacs; beyond the second or youngest of these latter is the epithelial process (enamel germ) of a third, and beyond this again (*f*) the forming enamel germ of a fourth. Thus, in a single section we have a perfected tooth and four stages in the formation of teeth exemplified.

FIG. 7.—Section of lower jaw of a young dog-fish.

Two only of the teeth seen in the section were exposed and in use; those lower down would be covered in by the fold of mucous membrane (*k*) (which in the section has been accidentally torn away and displaced to the right). The enamel organs form a continuous chain, each new one being derived from its immediate predecessor.

FIG. 8.—Section of lower jaw of a mackerel.

The developing teeth are lodged in a groove in the thin edge of the jaw; the perfected tooth is fixed in place by a sort of bony scaffolding, thrown out from the sides and bottom of this groove.

FIG. 9.—Two tooth-sacs of the common eel.

FIG. 10.—Tooth-sac of an eel more highly magnified.

The large cells of the enamel organ are confined to the region where the enamel cap is being formed; below this the enamel-cells are dwindled and insignificant.

FIG. 11.—Tooth-sac of a newt.

This is entirely cellular, so that under pressure it readily breaks up, nothing but cells remaining. The enamel organ consists principally of the enamel-cells (internal epithelium), the external epithelium being only feebly developed, and the stellate reticulum being wholly absent.

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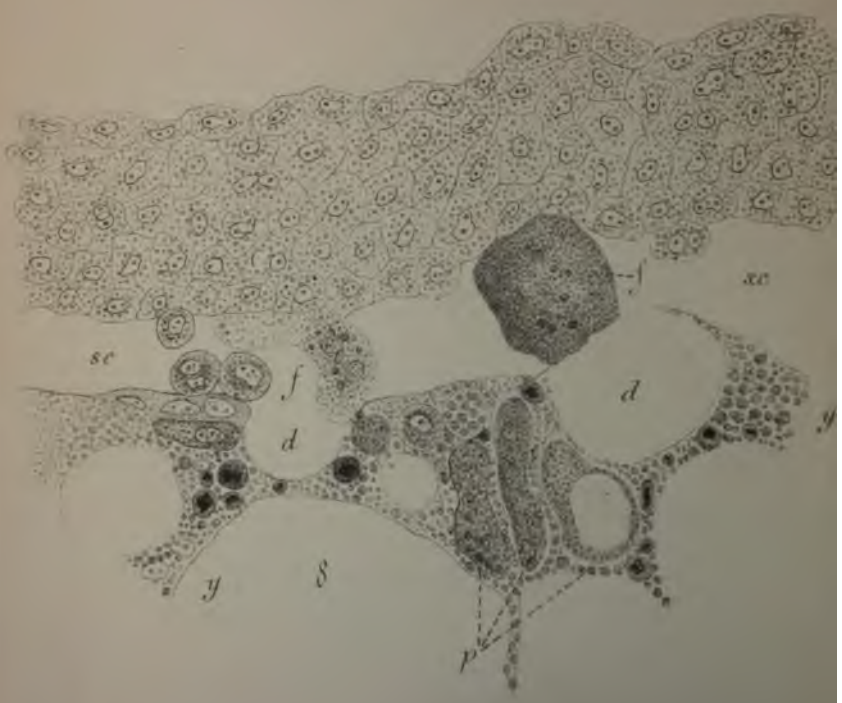
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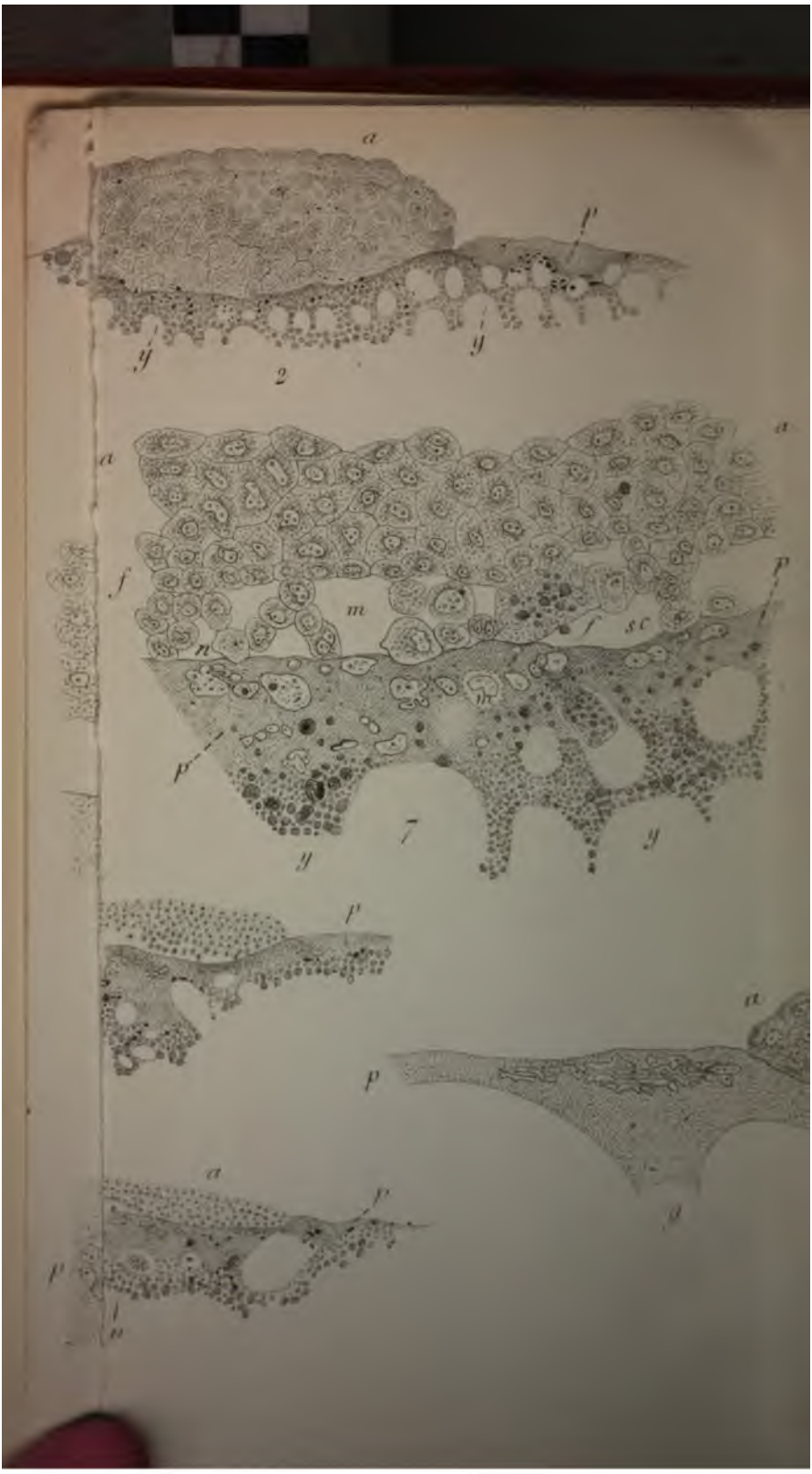
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EXPLANATION OF PLATE VI,

Illustrating Dr. E. Klein's Observations on the Early Development of the Common Trout.

FIG. 1.—Vertical section through a blastoderm 3 days old.
FIG. 2.—Similar section through a blastoderm 8 days old.
FIG. 3.—Vertical section through a blastoderm 9 days old.
FIG. 4.—12 days old.
FIG. 5.—13 days old.
FIG. 6.—14 days old.
} The section does not comprise the centre of the blastoderm.

FIG. 7.—Vertical section through an excentric portion of blastoderm 13 days old.

FIG. 8.—Vertical section through the same blastoderm as in the preceding figure, only nearer to the centre.

FIG. 9.—Vertical section through the very edge of a blastoderm 14 days old.

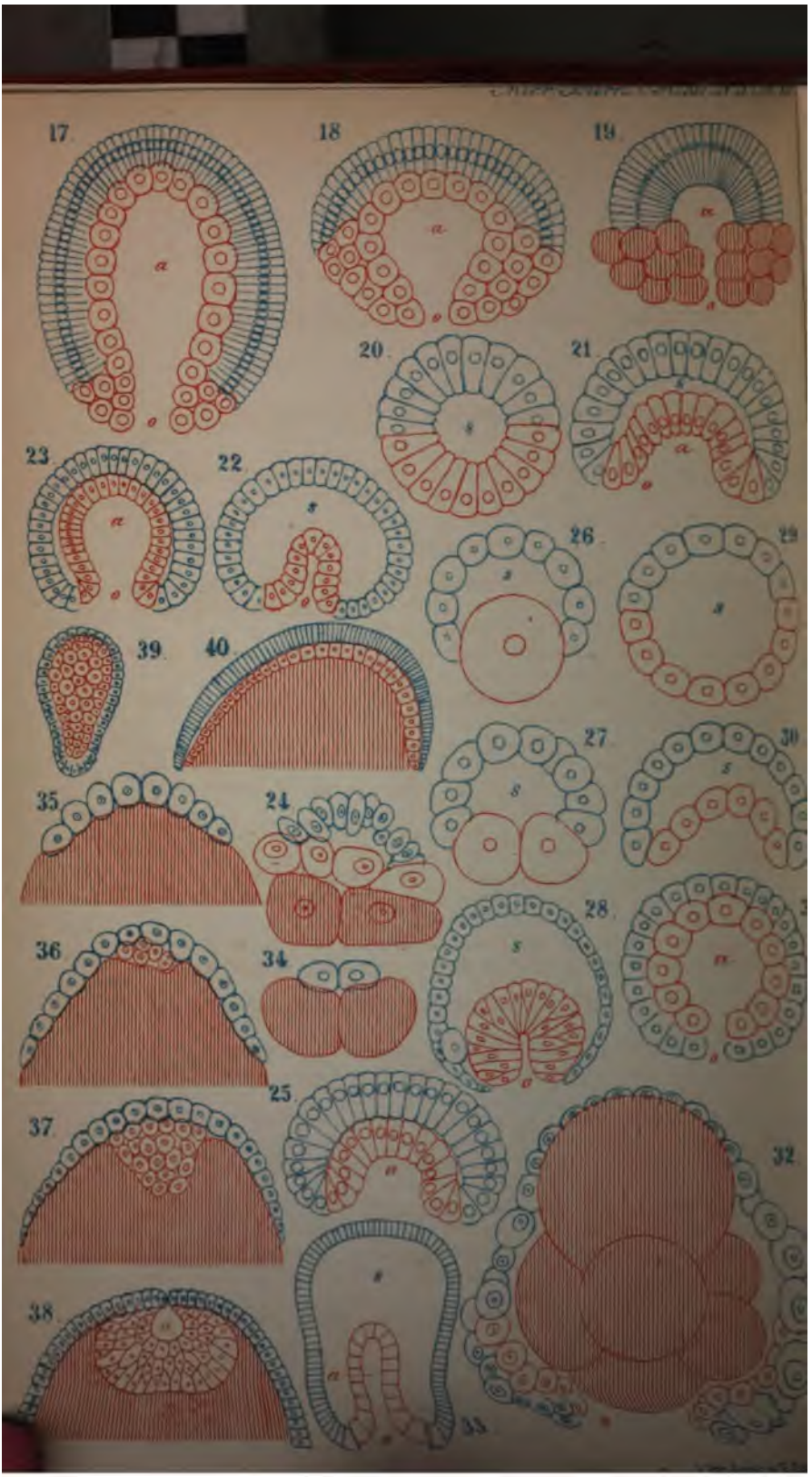
FIG. 10.—From a vertical section through an excentric portion of parablast of a blastoderm 13 days old.

All figures were drawn with camera lucida.

FIGS. 1, 2, 3, 4, 5, 6	with Hartnack's	Oc. ii, Obj. 4.
FIG. 7	,,	,,	Oc. ii, Obj. 8.
FIGS. 8, 9, and 10	,,	,,	Oc. iii, Obj. 8.

The meaning of the letters is as follows:

a. Archiblast.
p. Parablast.
y. Yolk.
sc. Segmentation cavity.
s. Surface of parablast.
f. Ordinary sized elements on the floor of segmentation cavity.
f. Large elements of the same kind.
n. Nucleus.
m. Transparent cells derived from the parablast.
d. Cavities in the yolk (fat-globules?).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EXPLANATION OF PLATES VII, VIII, IX, & X,

Illustrating the Notice of Professor Haeckel's recent additions to the Gastræa-Theory.

PLATE VII.

In all the figures the Entoderm is coloured red, the Exoderm blue. The food-yelk is mostly coloured red; *s*, cleavage-cavity (blastocœl); *a*, cavity of the primitive enteron; *o*, blastopore (Urmund).

Fig. 17.—Archigastrula of a Calcareous Sponge (*Asculmis armata*). After Haeckel, 'Monographie der Kalkschwämme,' Taf. 13, fig. 6.

Fig. 18.—Amphigastrula of a Calcareous Sponge (*Sycissa Huxleyi*). After Haeckel, 'Monog. d. Kalkschwämme,' Taf. 44, fig. 15.

Fig. 19. Amphiblastula of a Calcareous Sponge (*Sycandra raphanus*). After Oskar Schmidt ('Zeitschr. fur Wissen. Zool.,' Vol. xxv, Suppl., Taf. 9, fig. 5). The blastocœl or cleavage-cavity is by mistake marked with an *a* instead of with *s*. *o* should be omitted.

Fig. 20.—Archiblastula of a Coral (Actinia). After Kowalewsky (Russian memoir on the ontogeny of the Cœlenterata, Moscow, 1873, Plate iv, fig. 41).

Fig. 21.—Archigastrula of the same Coral (Ibid., Plate iv, fig. 2).

Fig. 22.—Archigastrula of a Medusa (Pelagia). After Kowalewsky (Ibid., Plate iii, fig. 2).

Fig. 23.—Archigastrula of a Worm (Sagitta). After Kowalewsky ('Embryologische Studien an Wurmen und Arthropoden,' Petersburg, 1871. Taf. 1, fig. 2).

Fig. 24.—Amphiblastula of a Worm (Euaxes). After Kowalewsky (Ibid., Taf. iv, fig. 27).

Fig. 25.—Archigastrula of a Brachiopod (Argiope). After Kowalewsky (Russian memoir on the ontogeny of the Brachiopods, Moscow, 1874, Plate i, fig. 3).

Fig. 26.—Amphiblastula of a Mussel (Unio). After an unpublished drawing of Carl Rabl.

Fig. 27.—Amphiblastula of the same Mussel in a later stage. After Carl Rabl.

Fig. 28.—Amphigastrula of the same Mussel. After Carl Rabl. On the left side is seen a large mesoderm-cell.

Fig. 29.—Archiblastula of a Snail (Limnæus). After Carl Rabl ("Die Ontogenie der Süsswasser," Pulmonaten 'Jenaische Zeitschrift für Naturw,' 1875, Vol. ix, Taf. vii, fig. 9).

Fig. 30.—Archiblastula invaginata of the same Snail. After Carl Rabl (Ibid., Taf. vii, fig. 10).

EXPLANATION OF PLATE VII.

FIG. 31.—Archigastrula of the same Snail. After Carl Rabl (Ibid., Taf. vii, fig. 11).

FIG. 32.—Amphigastrula of a Snail (Purpura). After Selenka ('Keimblätter bei Purpura,'' 'Niederl. Archiv f. Zool.,' 1871, Heft ii, Taf. xvii).

FIG. 33.—Archigastrula of a Sea-star (Asteracanthion). After Alexander Agassiz ('Embryology of the Star-fish,' 1864, Plate i, fig. 27).

FIG. 34.—Amphimorula of a Rhizocephalon (Sacculina). After Ed. Van Beneden ('Recherches sur l'Embryologie des Crustacées,' 1870, Plate i, fig. 21).

FIG. 35.—Discomorula of a Slater (Oniscus). After Bobretzky ("Zur Embryologie des *Oniscus murarius*," 'Zeitschr. fur Wissensch. Zoologie,' Vol. xxiv, Taf. xxi, fig. 3).

FIG. 36.—Discoblastula of the same Oniscus. After Bobretzky (Ibid., fig. 5).

FIG. 37.—Discogastrula of the same Oniscus. After Bobretzky (Ibid., fig. 7).

FIG. 38.—Discogastrula[1] of a Water-beetle (Hydrophilus). After Kowalewsky ('Embryologie Studien an Wurmen und Arthropoden,' Petersburg, 1871, Taf. ix, fig. 23).

FIG. 39.—Archigastrula of a Pteromaline (Platygaster). After Ganin ("Entwick. der Insecten," 'Zeitschr. f. Wiss. Zoologie,' 1869, Bd. xix, Taf. xxx).

FIG. 40.—Discogastrula of the Scorpion. After Metschnikoff ("Embryol. des Scorpinons," 'Zeitschr. f. Wiss. Zool.,' 1871, Taf. xiv, fig. 9).

PLATE VIII.

In all the figures the Entoderm is marked red, the Exoderm blue. The food-yelk is mostly coloured red. *s*, Cleavage-cavity (blastocœl); *a*, cavity of the primitive enteron; *o*, blastopore (Urmund of Haeckel).

FIG. 41.—Archiblastula of the Amphioxus. After Kowalewsky ("Entwick. des Amphioxus," 'Mem. Petersburg Akad.,' 1867, Vol. xi, Taf. i, fig. 9).

FIG. 42.—Archiblastula invaginata of the Amphioxus. After Kowalewsky (Ibid., fig. 13).

FIG. 43.—Archigastrula of the Amphioxus at its commencement. After Kowalewsky (Ibid., fig. 14).

FIG. 44.—Archigastrula of the Amphioxus completed. After Kowalewsky (Ibid., fig. 16).

FIG. 45.—Amphimorula of Petromyzon. After Max Schultze ('Entwick. von Petromyzon,' Haarlem, 1856, Taf. iv, fig. 1).

FIG. 46.—Amphiblastula of Petromyzon. After Max Schultze (Ibid., fig. 2).

FIG. 47.—Amphigastrula of Petromyzon at its commencement. After Schultze (Ibid., fig. 5).

FIG. 48.—Amphigastrula of Petromyzon, complete. After Schultze (Ibid., fig. 7).

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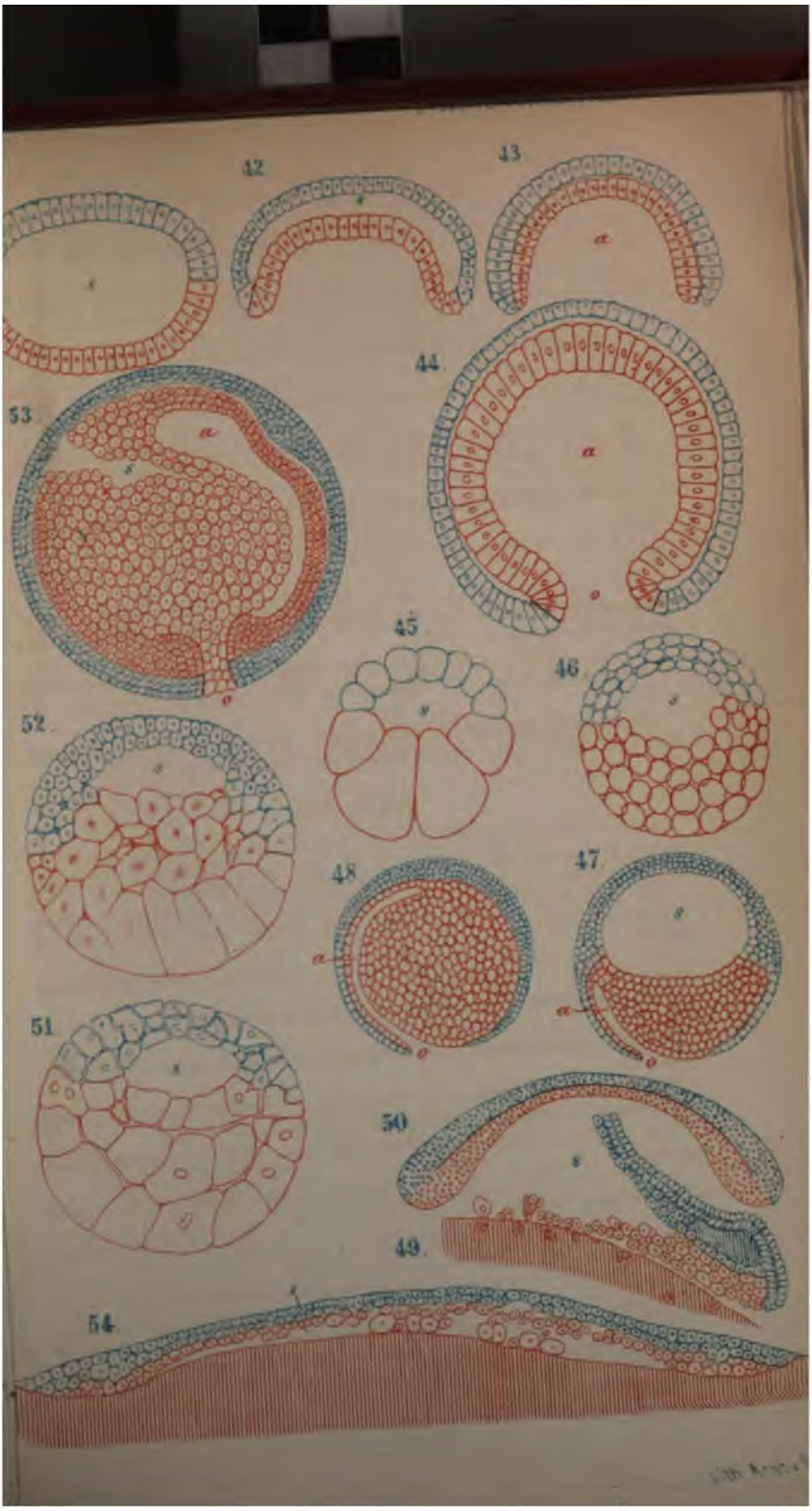
[1] It has not been my intention to criticise all the interpretations which Haeckel has offered of developmental forms in the present notice, but I must express my difficulty in interpreting the ontogeny of Hydrophilus as belonging to the Discoblastic type. It seems to me rather to be a very much [illegible] form.—E. R. L.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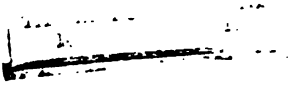

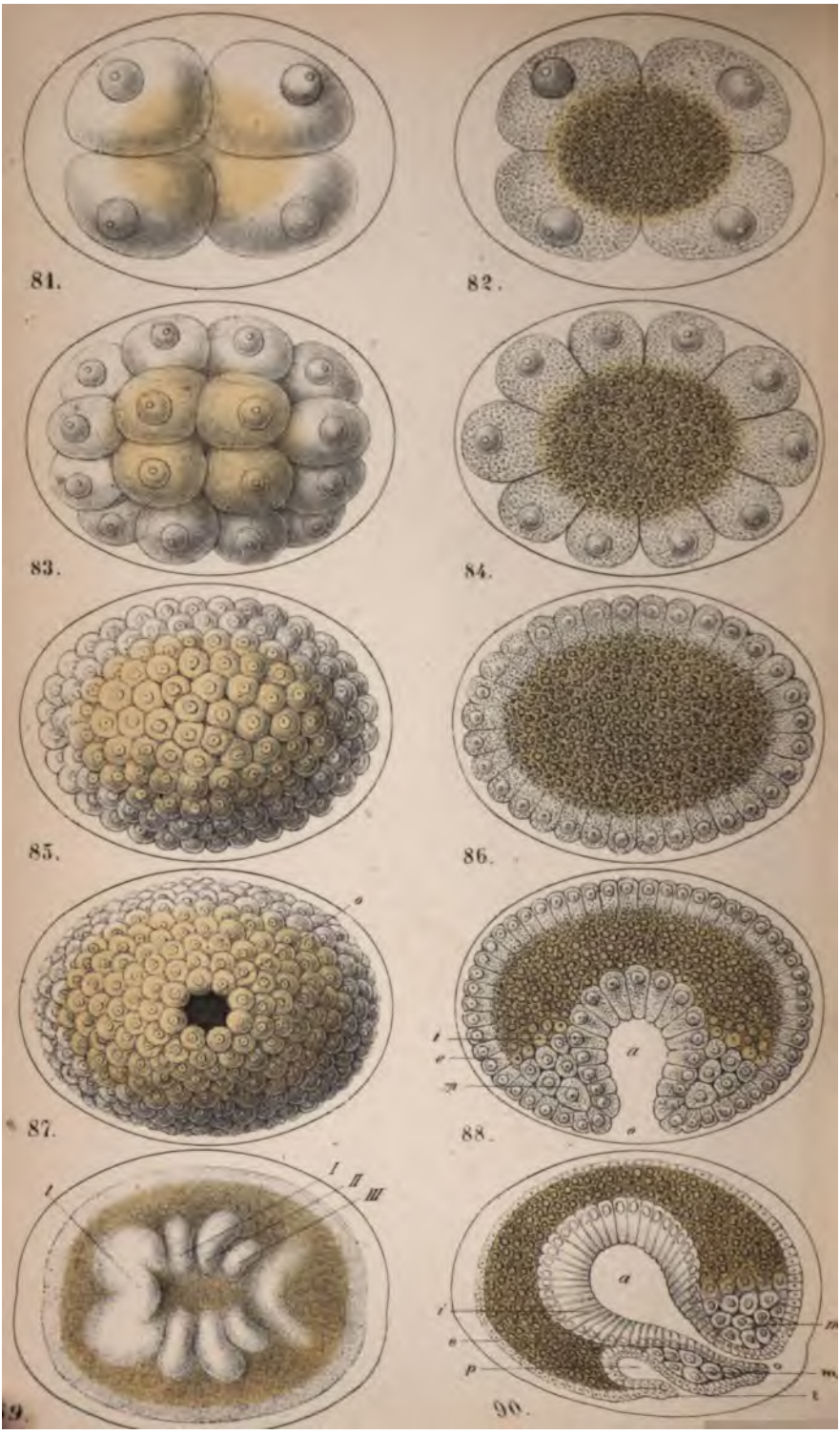
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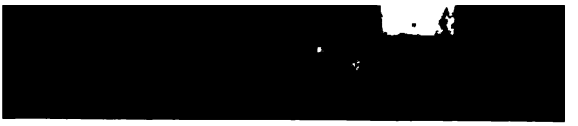

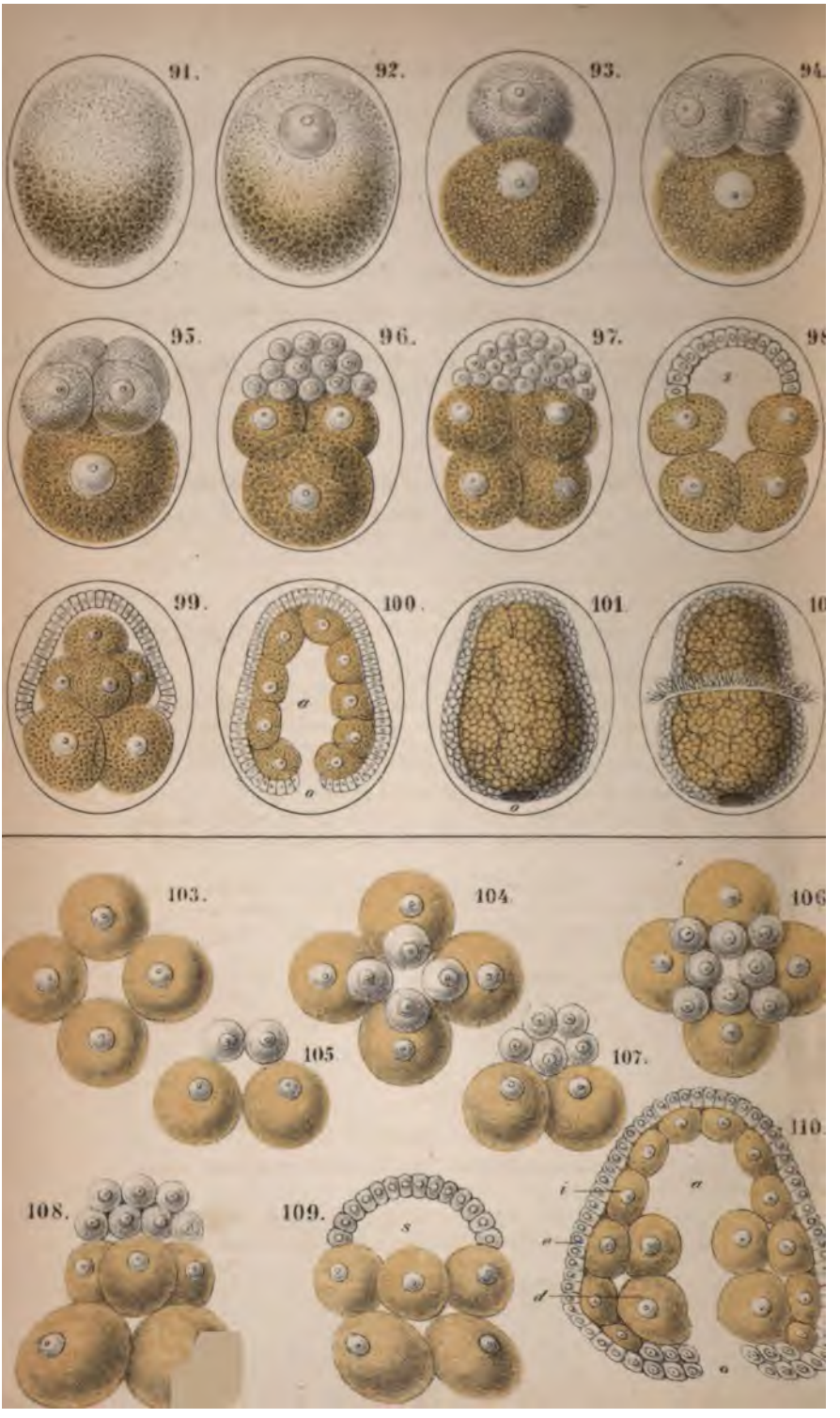
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EXPLANATION OF PLATE IX & X.

Fig. 49.—Discogastrula of a Shark (Mustelus). After Balfour ("Development of the Elasmobranch Fishes," 'Quart. Journ. Microsc. Science,' 1874, Vol. xiv, Plate xiii, fig. 1).

Fig. 50.—Discogastrula of a Boney Fish (Esox). After Lereboullet ('Recherches d'Embryol. comparée,' 1853, Plate i, fig. 27).

Fig. 51.—Amphimorula of Bombinator. After Goette ('Entwick. der Unke,' 1875, Taf. ii, fig. 27).

Fig. 52.—Amphiblastula of Bombinator. After Goette (Ibid., Taf. ii, fig. 28).

Fig. 53.—Amphigastrula of Bombinator. After Goette (Ibid., Taf. ii, fig. 33).

Fig. 54.—Discogastrula of the Chick. After Goette ("Die Bildung der Keimblätter im Huhnerei," 'Archiv f. Mikrosk. Anatomie,' Vol. x, 1874, Taf. x, fig. 4).

PLATE IX.

Superficial cleavage and Perigastrula of a Crustacean (Peneus).

Fig. 81.—Second cleavage-phase: egg with four cleavage-cells (blastomeres, Huxley) seen from the surface. Four cells have been separated by two annular grooves, an equatorial and a meridianal, passing at right angles to one another, whilst the central food-yelk remains undivided.

Fig. 82.—The same egg seen in meridianal section.

Fig. 83.—Fifth cleavage-phase: egg with 32 blastomeres, seen from the surface.

Fig. 84.—The same egg in meridianal section.

Fig. 85.—Perimorula (and at the same time Periblastula), seen from the surface. After the completion of the cleavage-process the blastomeres form on the surface of the egg, a single compact layer of equiformal cells (blastoderm), which encloses the internal uncleft food-yelk.

Fig. 86.—The same Perimorula in meridianal section.

Fig. 87.—Perigastrula, seen from the surface; in the middle is the 'Urmund' (blastopore), *o*, which leads into the invaginated 'Urdarm' (archenteron).

Fig. 88.—The same Perigastrula in section. *a*, Urdarm (archenteron), and *o*, Urmund (blastopore); *e*, exoderm; *i*, entoderm; *m*, mesoderm.

Fig. 89.—Nauplius-stage, seen from the ventral surface. *l*, Upper-lip; I, II, III, rudiments of the first three pairs of appendages,

Fig. 90.—The same nauplius-stage in fore-and-aft vertical section (seen from the left side). *a*, Archenteron; *o*, blastopore; *m*, mesoderm-cells; *l*, upper-lip; *p*, invagination of the pharynx and gizzard (stomodæum, L.); *e*, exoderm; *i*, entoderm.

PLATE X.

Unequal cleavage and Amphigastrula.

Figs. 91—102.—Unequal cleavage and Amphigastrula of a Chætopodous Annelid (Fabricia).

—Amphimonerula. After fertilization the germinal vesicle has
and a cytod has arisen from the copulation of sperm-cell and

—tula, the first cleavage-sphere.

EXPLANATION OF PLATE X—*Continued.*

FIG. 93.—First cleavage-phase: the Amphicytula has divided into tw cells, an upper, smaller, and clearer animal cell (mother-cell of the exoderm) and a lower larger, and darker vegetative cell (mother-cell of the entoderm).

FIG. 94.—Second cleavage-phase: the upper animal cell has divided int two cells; the lower vegetative cell is undivided.

FIG. 95.—Third cleavage-phase: the upper animal cell is divided into four cells by two meridianal grooves. The lower vegetative cell is still uncleft.

FIG. 96.—Later cleavage-phase: the upper animal cell has broken up into several small clear cells; the lower vegetative cell has divided into three large dark cells (a lower larger and two upper smaller).

FIG. 97.—Amphimorula. After the conclusion of the cleavage-process, there is found above at the animal pole a hemispherical mass of numerous small clear cells (exoderm); below, at the vegetative pole, a dark mass of a few (six ?) large dark cells (entoderm).

FIG. 98.—Amphiblastula in meridianal section. In the interior a cleavage-cavity (*s*) has formed by accumulation of fluid; in the upper (animal) half, roofed in by a hemispherical layer of small clear exoderm-cells; in the lower (vegetative) half, closed by a few large dark entoderm-cells.

FIG. 99.—Amphiblastula in the course of invagination or over-growth (invaginata-circumcreta). The large dark entoderm-cells become pushed in to the cleavage-cavity and *eo ipso* grown over by the clear exoderm-cells. Optical meridianal section.

FIG. 100.—Amphigastrula, in optical meridianal section. The invagination (entobole) or over-growth (epibole) of the Amphiblastula is finished, the cleavage-cavity has disappeared, and the cavity of the archenteron is formed. *o*, Urmund (blastopore).

FIG. 101.—The same Amphigastrula, seen from the surface.

FIG. 102.—Young worm-larva with a ring of cilia.

FIGS. 103.—110.—Unequal cleavage and Amphigastrula of a Snail (Trochus ?)

FIG. 103.—Second cleavage-phase. Egg with four cleavage-cells.

FIG. 104.—Third cleavage-phase. Egg with eight cleavage-cells (four small, clear animal cells, and four large, dark vegetative cells).

FIG. 105.—The same egg in profile.

FIG. 106.—Fourth cleavage-phase. Egg with twelve cleavage-cells (eight small, clear animal cells, and four large, dark vegetative cells).

FIG. 107.—The same egg in profile.

FIG. 108.—Amphimorula in meridianal section. After complete cleavage, the upper animal hemisphere of the egg consists of sixteen small clear cells; the lower vegetative hemisphere, on the other hand, consists of eight large dark cells (four upper larger, and four lower and smaller).

FIG. 109.—Amphiblastula in meridianal section. The roof of the cleavage-cavity is formed by thirty-two small clear cells, its floor by eight large dark cells (four upper small, and four lower large).

FIG. 110.—Amphigastrula in meridianal section. *a*, Urdarm (archenteron); *o*, Urmund (blastopore). The exoderm (*e*) has completely grown over the entoderm (*i*). Most of the cells of the last form the wall of the enteron. A few large cells of the entoderm remain over and above as 'food-yelk' (*d*).

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

Fig. 14.

Fig. 1.

Fig. 2.

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Fig. 5.

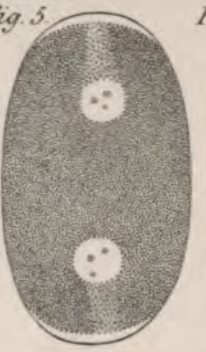

Fig. 6.

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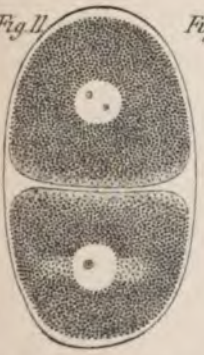


Fig. 12.

Fig. 13.

Fig. 14.

EXPLANATION OF PLATES XI AND XII,

Illustrating Mr. John Priestley's Paper on Recent Researches on the Nuclei of Animal and Vegetable Cells, and especially of Ova.

PLATE XI.

(*The Figures are from Auerbach's 'Organologische Studien.'*)

Fig. 1.—Nuclei and cells from the carp's liver.

a—*d*. Nuclei in their natural condition. *e*—*g*. Isolated nuclei in the condition of water-shrinking, with expressed hyaline droplets. *l*. Internal swelling of nuclei. *h*, *i*. Isolated nuclei in concentrated solution of NaCl. *k*. Isolated nucleus in concentrated solution of acetic acid. *m*. Water-shrinking of nucleus within its cell.

Fig. 2.—Nuclei from the gastric and intestinal cylindrical epithelial cells of the frog.

A. Natural condition. B. Under the influence of very dilute solutions of NaCl. C. Under the influence of pure water.

The remaining figures represent the ova of *Ascaris nigrovenosa* in a strongly compressed condition.

Figs. 3—7.—Formation of yolk-membrane and primary cleavage-nucleus.

Figs. 8—11.—Formation of bistellate figure and segmentation into two.

Figs. 12, 13.—Segmentation into four. In the course of elongation and formation of the bistellate figure (fig. 12) the positions of the segments change, from superior and inferior, to supero-lateral and infero-lateral.

Fig. 21.—Exceptional elongation of the lower of the nuclei in fig. 11 in a direction with, instead of across, the long axis of the ovum, and consequent abnormal disposition of the four resulting segments.

PLATE XII.

(*The Figures are from Strasburger's work 'Ueber Zellbildung und Zelltheilung.'*)

FIGS. 1—6.—Free-cell formation in *Phaseolus multiflorus*. (Alcoholic preparations.)

1. Earliest beginnings of an endosperm-cell in the looser portion of the peripheral protoplasmic layer of the embryo-sac; the nucleus is still punctiform.

2—6. The same at later stages.

FIGS. 7—10.—Cell-division in *Picea vulgaris*.

FIGS. 11, 12.—Cell-division in *Pinus sylvestris*.

7. The four nuclei which appear at once in the embryonal vesicle after the original nucleus has vanished.
8. Optical section of the same part of the embryonal vesicle.
9. Preparations for division; in the left-hand nucleus the nuclear disc has already split into two.
10. A later stage than in 9; the equatorial cell-plates.
11. Immediately after the formation of the new nuclei; cell-plates well marked.
12. The cellulose membrane already separated out between the two laminæ of the cell-plate.

FIGS. 13—16.—Cell-division in *Phallusia mamillata*.

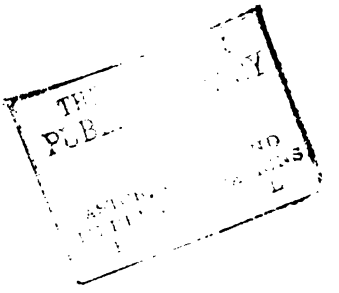
FIG. 17.—Cell-division in *Cucullanus elegans* (after Bütschli).

FIG. 18.—Cell-division in *Blatta germanica* (after Bütschli).

13, 14. Commencement and migration of the nucleus in fertilised ova (alcoholic preparations).
15. Elongation of nucleus and formation of nuclear disc (alcoholic preparation).
16. Division of nucleus (fresh preparation).
17. A dividing ovum. In the upper cell the nucleus is spindle-shaped and striated, the nucleus disc having just divided.
18. Nuclear plate or disc has just divided.

FIG. 19.—Development of new nuclei and formation of the division between the cells (*Ginkgo Viloba*).

FIG. 20.—Upper four cells of a hair from a stamen of *Tradescantia virginica*; the highest still dividing; the lower two have just arisen by division within their mother-cell.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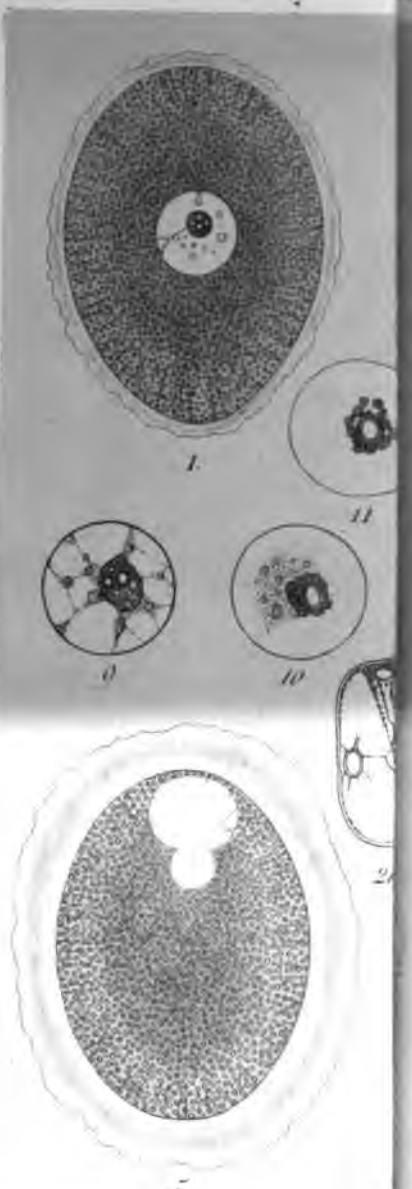
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EXPLANATION OF PLATE XIII,

Illustrating Edouard van Beneden's Contributions to the History of the Germinal Vesicle, and of the First Embryonic Nucleus.

FIGS. 1—19.—*Asteracanthion rubens.*

FIG. 1.—An ovarian egg arrived at maturity, but still showing its germinal vesicle at the centre of the yolk.

FIGS. 2—8.—Represent one and the same egg in which the different phases of the disappearance of the germinal vesicle have been observed. Subsequently I saw in the same egg the retraction of the yolk, the yolk assume an almost spherical form, the polar globules appear in the peri-vitelline liquid, the first embryonic nucleus appear in the centre of the yolk, and finally the yolk divide into two spheroids. I have not figured these last modifications, both because I have not described them in the text and because I have never been able to observe either how the directive bodies are formed or how the first embryonic nucleus is produced. I quote these later modifications as having been produced under my eyes in the same egg in which I traced the series of changes here figured (2—8), for this reason—that these facts prove that the egg in question was normal and developed in a normal manner, although its oval shape is exceptional for a ripe egg. All the other eggs in which I saw the germinal vesicle disappear were spherical or approximately so.

FIG. 2.—By the side of a nucleolus which has an uneven outline, and in which there is only a single vacuole, are still seen pseudo-nucleoli.

FIG. 3.—The pseudo-nucleoli have disappeared and the nucleolus appears composed of a certain number of globules collected round a clear transparent central corpuscle.

FIG. 4.—The nucleolus is broken up into a large number of fragments, the refractive power of which gradually diminishes, at the same time that the outline of the vesicle becomes less distinct.

FIG. 5.—The last traces of the nucleolus have disappeared. The membrane of the germinal vesicle has burst, and the contents escape by the opening.

FIGS. 6, 7.—The contents of the vesicle are in great part escaped, the membrane of the vesicle shrinks and the volume occupied by the germinal matter diminishes.

FIG. 8.—The last traces of the germinal vesicle have disappeared.

FIG. 9.—Germinal vesicle of an egg similar to that which I have represented in fig. 1, after its escape from the yolk. In the nucleoplastic network are seen the nucleolus and the pseudo-nucleoli. Observed with Hartnack's No. 9 immersion objective.

EXPLANATION OF PLATE XIII.—*Continued.*

FIGS. 10—16.—Represent the successive modifications undergone by the germinal vesicle of the same egg, as they were produced under my eyes.

FIG. 10.—Beside the nucleolus are seen pseudo-nucleoli suspended in a mass of granular matter (nucleoplasma).

FIG. 11.—The pseudo-nucleoli have disappeared. The nucleolus is divided into a large number of fragments, which rapidly disperse and spread through the whole extent of the germinal vesicle, as seen in figs. 12 and 13. The gradual diminution in the refractive power of the nucleolar substance is also noticeable.

FIG. 14.—The central part of the nucleolus alone remains visible.

FIG. 15.—All trace of nucleolus has disappeared.

FIG. 16.—The membrane is ruptured and the contents of the vesicle escape.

FIGS. 17, 18, 19.—Three successive phases of the disappearance of the germinal substance. These modifications were produced in the space of twelve minutes.

FIGS. 20, 21.—*Dicyema Elidones.*

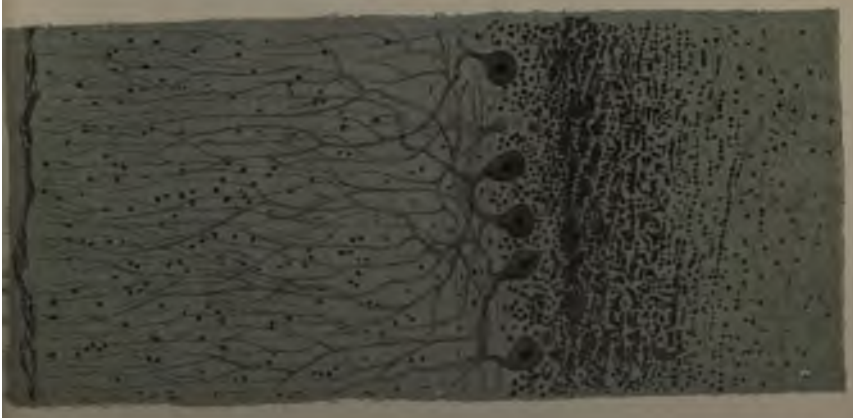
The two figures represent the nucleus of the central cell of the body of *Dicyema Elidones*. Kölliker and Wagner, regarding the body of these organisms as hollow, have called the supposed central cavity of the body by the name of *Leibeshöhle*.

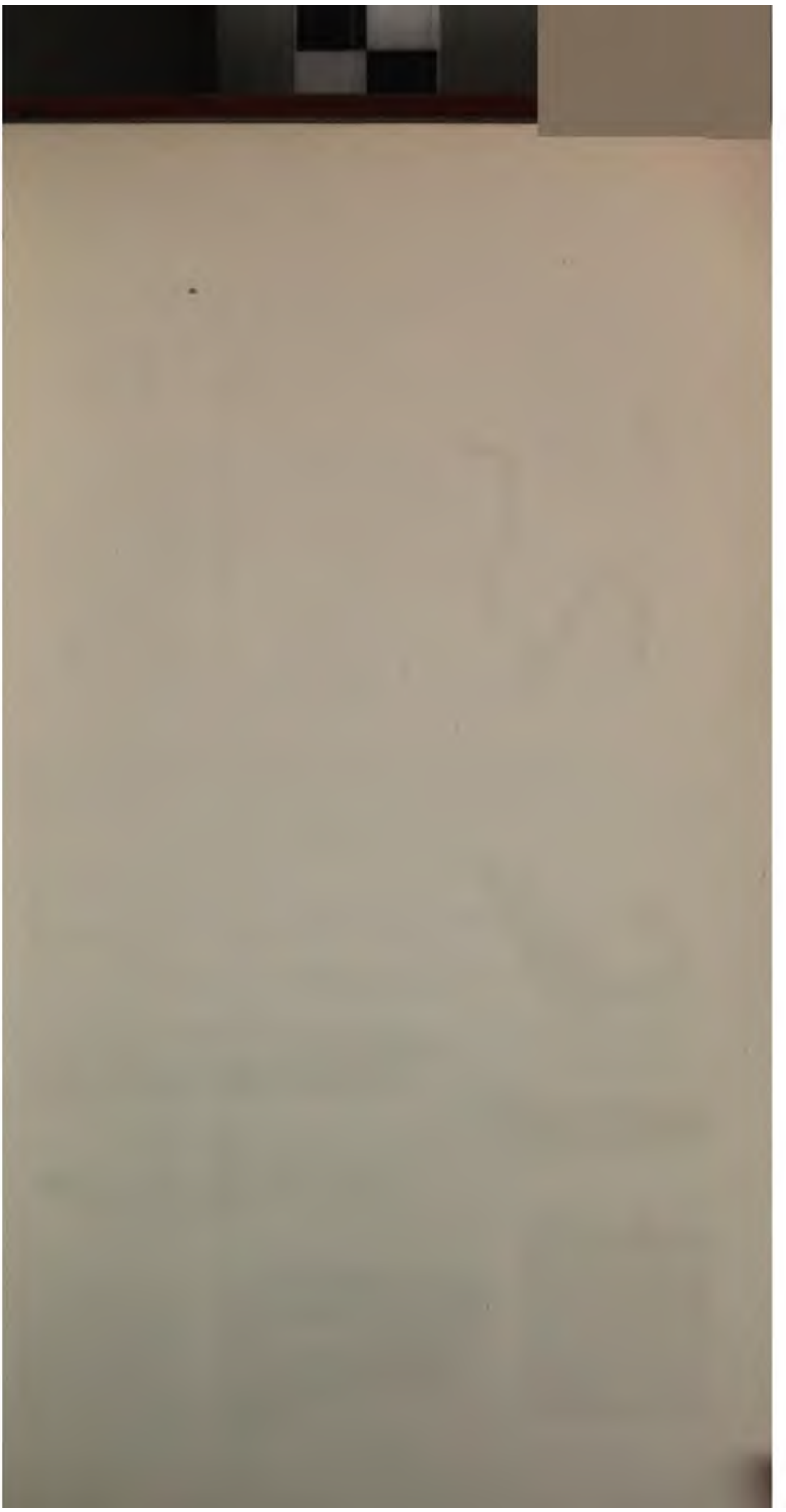
According to my observations, which have reference both to the organization and the development of Dicyema, there is no general body-cavity. The axis of the cylindrical body is occupied by an immense cell, which extends from the cephalic to the caudal extremity of the organism. In this cell, which is filled with clear hyaline contents and traversed by a protoplasmic network, the germs originate and embryos develop. It is the nucleus of this cell that I have figured.

FIG. 20.—Shows the nucleus seen in section. The nucleus is limited by a thick membrane. It is filled with a clear homogeneous, colourless and transparent substance; it is traversed by bands of nucleoplastic matter. On the left is the nucleolus formed of a thin peripheral layer and a large vacuole. Near the superior pole of the nucleus are seen large granulations (pseudo-nucleoli ?).

FIG. 21.—Represents the same nucleus viewed from above, to show the nucleoplastic network extended under the nuclear membrane. (Hartnack's No. 10 immersion.)

PLATE XIV illustrates Mr. Sankey's Memoir on a New Process for Examining the Structure of the Brain, and PLATE XIX that of Dr. Carpenter on the genus Astrorhiza of Sandahl.





EXPLANATION OF PLATE XV,

Figs. 6—14.

Illustrating Dr. Thin's paper on the Structure of Muscular Fibre.

(The figures were drawn as observed with Hartnack's Objectives No. 3 or No. 7, with No. 3 Eyepiece (old system). According to the values assigned to these numbers in the *Prix-Courant*, Paris, 1872, the No. 3 is equivalent to a magnifying power of 50, and the No. 7 of 300 diameters).

FIG. 6.—Primary bundle (*a*) isolated. Obj. 7.

FIG. 7.—Nuclei (*a*) shown applied to the surface of a primary bundle. Obj. 7.

FIG. 8.—Elongated narrow cells (*a*) partly detached from surface of a primary bundle. Obj. 7.

FIG. 9.—Cells seen entire on surface of secondary bundle. At *a* two cells have the position of the nuclei similar to that observed in tendon-cells. Obj. 7.

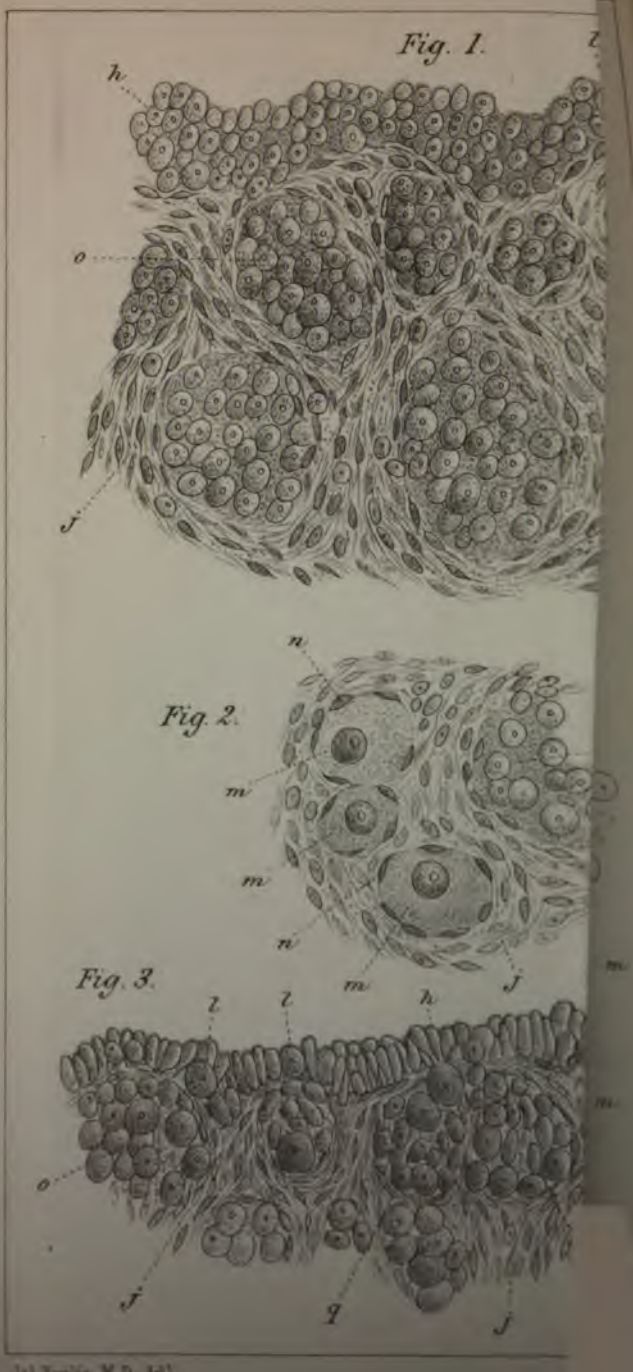
FIG. 10.—An unbroken muscular fibre showing a natural divison at one end into the larger component bundles. Obj. 3.

FIG. 11.—Part of the fibre shown in fig. 10 more highly magnified. Obj. 7.

FIG. 12.—Divided end of a fibre in which the nuclei are well seen. Obj. 7.

FIG. 13.—The sarcolemma (*b*) preserved and within it a secondary bundle. Obj. 3.

FIG. 14.—The same as fig. 13, more highly magnified. (*a*) Sarcolemma with nuclei, (*b*) the secondary bundle. Obj. 7.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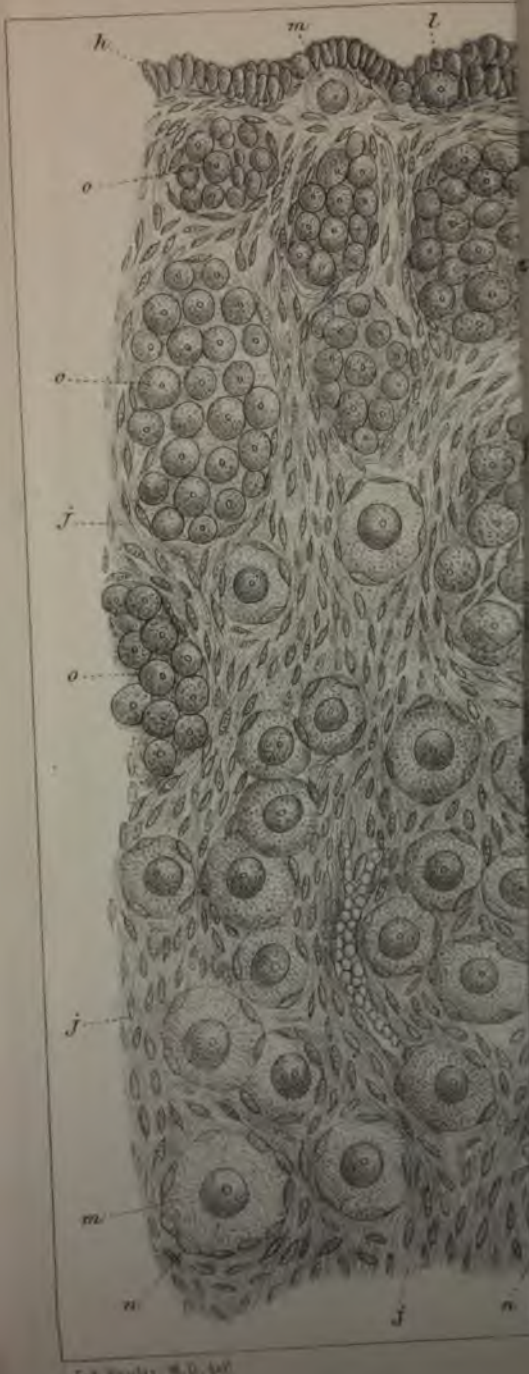


Fig. 8.

Fig. 9.

Fig. 11.

Fig. 14.

Jas Fenlis. M.D. del.

Fig. 15.
m
n
n'
n
j

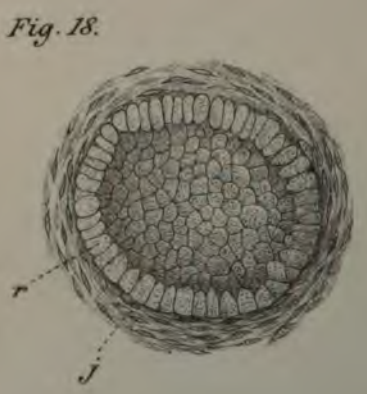


Fig. 18.
r
j

u
Fig. 20.
r
j

EXPLANATION OF PLATES XVI, XVII, XVIII,

Illustrating Dr. Foulis's paper on the Development of the Ova and Structure of the Ovary in Man and other Mammalia.

In these plates the same letters have been employed to mark corresponding structures in the whole series of figures.

h. Germ epithelial corpuscles.
h_1. Corpuscles from the surface of adult ovary.
j. Connective tissue stroma of the ovary.
k. Blood-vessels.
l. Large spherical germ epithelial corpuscles.
m. Primordial ova.
n. Fusiform corpuscles in contact with the yelk substance of the primordial ova.
o. Large egg clusters.
p. Tubiform depressions of the surface of the ovary.
q. Small groups of germ epithelial corpuscles being included in the stroma of the ovary.
r. The membrana granulosa.
s. The zona pellucida.
t. The yelk substance of the nearly mature ovum.
u. Individual corpuscles of the membrana granulosa, showing the nucleus surrounded with a considerable quantity of protoplasm.
v. Division of the nuclei of the corpuscles of the membrana granulosa.

All the figures were drawn by the aid of the camera lucida from actual preparations.

With the exception of figures 3, 5 and 24, R. and J. Beck's one-tenth immersion lens, adapted to Hartnack's instrument with ocular No. 3, was used.

In figures 3 and 24 ocular No. 4 was employed.

In figure 5 objective one sixth (Swift) with ocular No. 3.

PLATE XVI.

Fig. 1.—A portion of the ovary of a human fœtus of three and a half months, showing the germ epithelium (*h*) and groups of large germ epithelial corpuscles or egg clusters (*o*) imbedded in the stroma. The stroma (*j*) consists almost entirely of connective tissue corpuscles.

Fig. 2.—A portion of the same ovary, showing large primordial ova (*m*) imbedded in the stroma (*j*). Fusiform corpuscles (*n*) of the stroma are seen in contact with the yelk of these primordial ova.

Fig. 3.—A portion of the ovary of a human fœtus of seven and a half months, showing the manner of inclusion of the germ epithelial corpuscles in groups (*q*) in the stroma of the ovary.

Fig. 4.—A portion of the ovary of a human fœtus of seven and a half months, showing in section the furrows (*p*) which lie between the prominences on the surface of the ovary.

Fig. 5.—A portion of the ovary of a child at birth, showing the stroma (*j*) crowded with primordial ova (*m*).

PLATE XVII.

Fig. 6.—Section through the ovary of a human fœtus of seven and a half months, showing the germ epithelium (*h*), large spherical corpuscles (*l*), and groups of similar corpuscles or egg clusters (*o*) imbedded in meshes of the stroma (*j*). In the lower part of the figure many primordial ova (*m*) in various stages of development are seen. In contact with each primordial

ovum are fusiform connective tissue corpuscles (*n*), similar to the fusiform corpuscles of which the stroma consists. Numerous blood-vessels (*k*), whose walls consist of connective tissue corpuscles, ramify throughout the whole ovary.

FIG. 7.—A small egg cluster consisting of large spherical germ epithelial corpuscles with fusiform connective tissue corpuscles (*n*) intermingled.

FIG. 8.—A small group of spherical germ epithelial corpuscles.

FIG. 9.—A primordial ovum. It has no zona pellucida.

FIGS. 10, 11.—Primordial ova (*m*). In contact with and indenting the yelk substance of each, are fusiform connective tissue corpuscles from the wall of the young Graafian follicle.

FIG. 12.—The membrana granulosa (*r*) when first formed seen in section. Outside the membrana is the ovarian stroma (*j*). From the human ovary at birth.

FIG. 13.—The corpuscles of the membrana granulosa from the nearly ripe Graafian follicle of the rabbit's ovary. The letter *v* points to the division of the nuclei of the corpuscles.

FIG. 14.—Surface view of the membrana granulosa (*r*) when first formed. From the human ovary at birth.

PLATE XVIII.

FIGS. 15, 16, 17, 18, 19, 20, illustrate the development of the membrana granulosa in the ovary of an adult rabbit.

FIG. 15.—A primordial ovum (*m*). Fusiform connective tissue corpuscles (*n*) of the stroma (*j*) lie in contact with the yelk substance of the young ovum.

FIG. 16.—A young ovum farther advanced in development than the last. Some of the fusiform corpuscles (*n*) in contact with the yelk are swollen out.

FIG. 17.—An ovum still farther advanced in development, completely surrounded by a wreath of corpuscles, which have been derived from the fusiform corpuscles of the stroma (*j*). This wreath is the membrana granulosa (*r*) seen in section.

FIG. 18.—A Graafian follicle from which the ovum has been removed The membrana granulosa (*r*) is seen in section.

FIG. 19.—A section through a Graafian follicle and the nearly ripe ovum contained in it. The membrana granulosa (*r*) consists of several layers of corpuscles. The zona pellucida (*s*) is well developed, and the yelk substance (*t*) at its peripheral part contains numerous bright granules and particles.

FIG. 20.—A section through the membrana granulosa and wall of a ripe Graafian follicle just before bursting. The membrana granulosa (*r*) consists of several layers of corpuscles. Each corpuscle is a large nucleus surrounded by protoplasm (*u*). In the wall of the follicle outside the membrana granulosa the fusiform corpuscles of the stroma (*j*) are very large.

FIG. 21.—A section through the egg zone of an old cat's ovary. The stroma (*j*) consists almost entirely of fusiform corpuscles. Around the young eggs (*m*) the fusiform corpuscles (*n*) of the stroma may be traced in their development into the corpuscles of the membrana granulosa.

FIG. 22.—The epithelium (h_1) from the surface of the same cat's ovary.

FIG. 23.—The epithelium (h_1) from the surface of the ovary of a child six years of age.

FIG. 24.—The same epithelium more highly magnified showing the "grooved" appearance of the corpuscles.

FIG. 25.—Germ epithelial corpuscles (*h*) seen in profile. From the ovary of a seven and a half months' human fœtus.

FIG. 26.—A primordial ovum (*m*) surrounded with fusiform corpuscles (*n*). From the stroma of the same ovary.

FIG. 27.—An ovum (*t*) from the deeper parts of the same ovary, showing the appearance of the membrana granulosa (*r*) when first formed round the ovum.

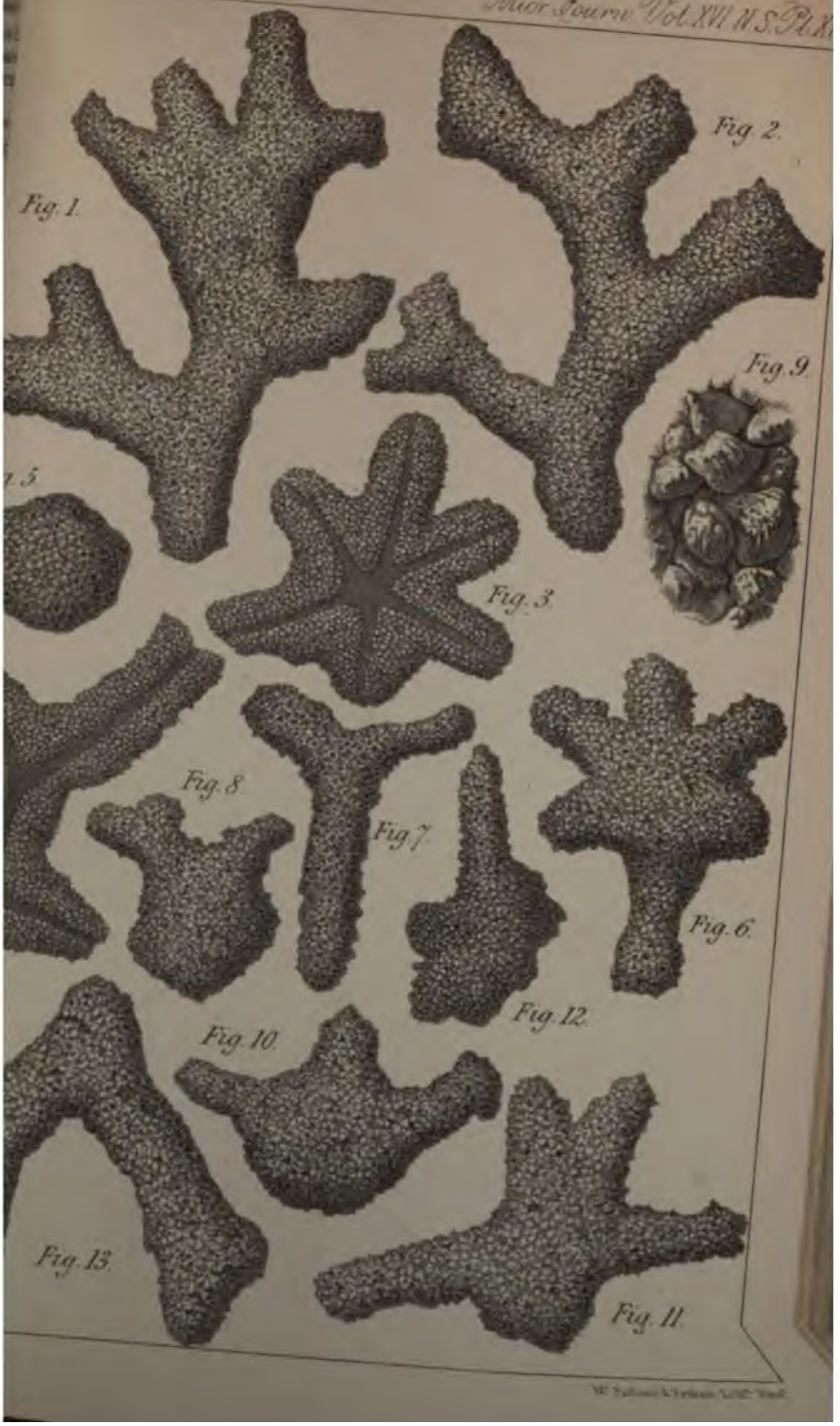
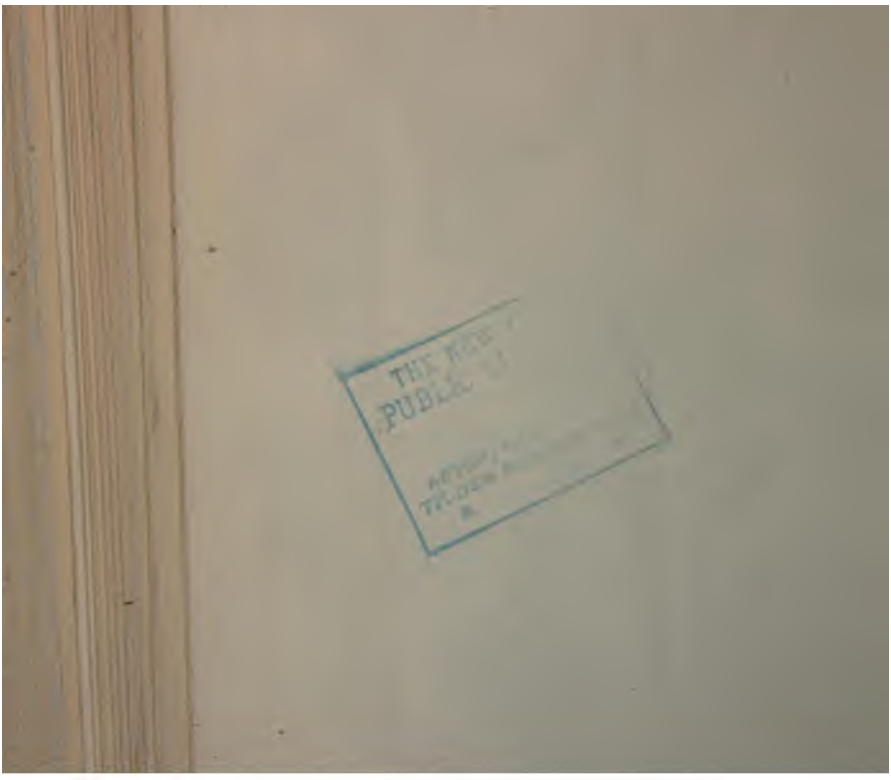


Fig. 1.
Fig. 2.
Fig. 9.
Fig. 3.
Fig. 8.
Fig. 7.
Fig. 6.
Fig. 12.
Fig. 10.
Fig. 13.
Fig. 11.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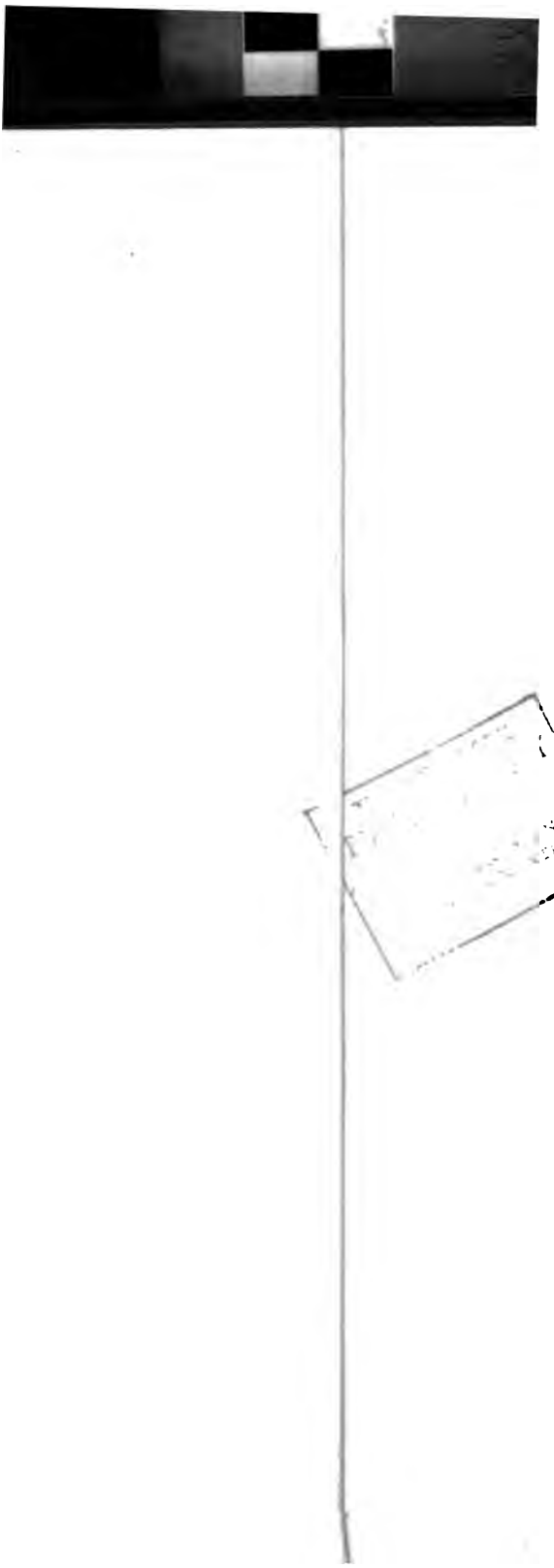
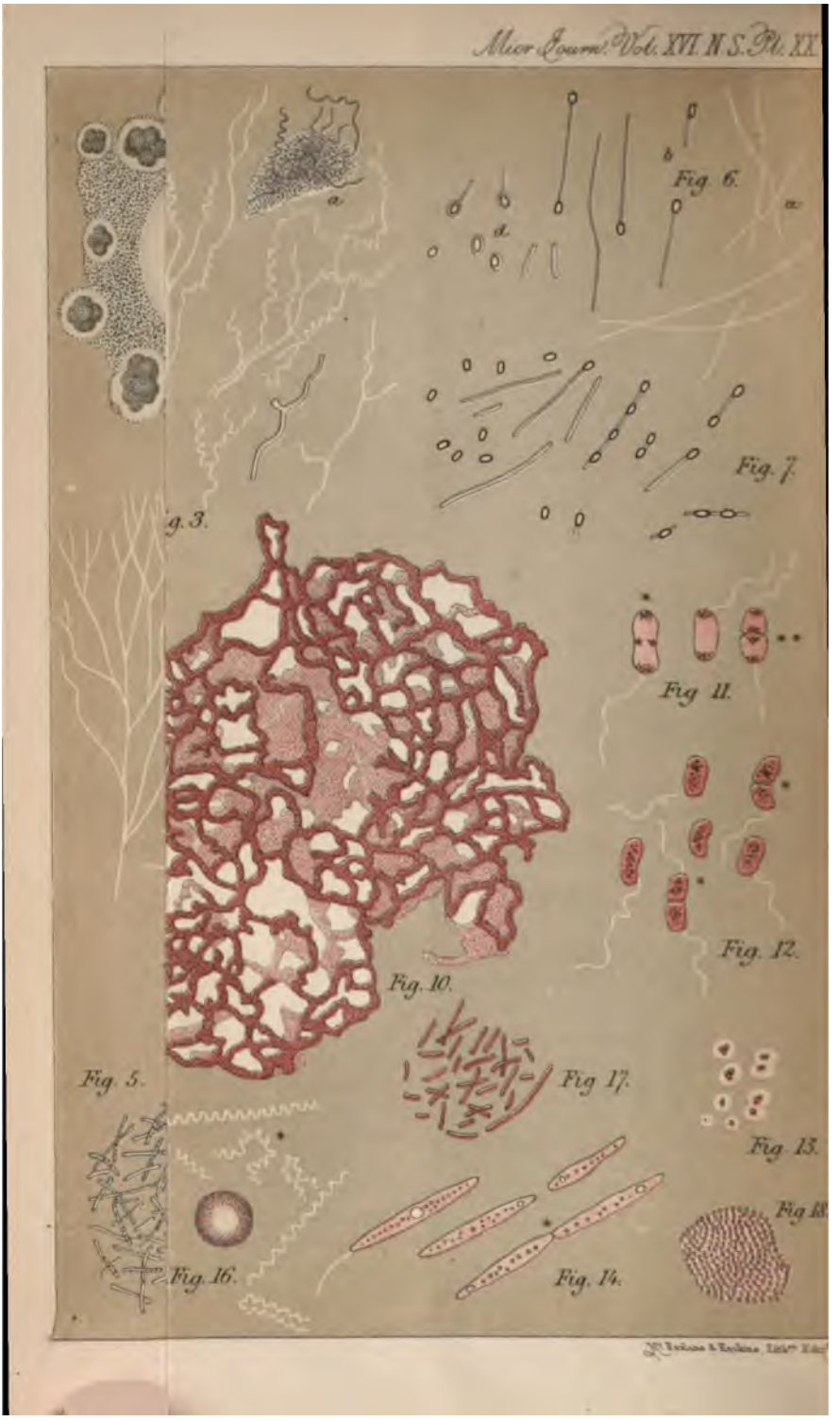



a
b
d
Fig. 6.
Fig. 7.
Fig. 3.
Fig. 11.
Fig. 12.
Fig. 10.
Fig. 5.
Fig. 17.
Fig. 13.
Fig. 16.
Fig. 14.

EXPLANATION OF PLATE XX,

Illustrating Mr. Jeffrey Bell's Account of Recent Researches into the History of Bacteria, made by and under the direction of Professor Cohn.

FIG. 1.—*Ascococcus Billrothii*, large knob-like cell-families, surrounded by smaller ones, and embedded in *Micrococcus*.

FIG. 2.—*Myconostoc gregarium:* Gelatinous spheroids, in which are fibres irregularly placed and rolled together. *a*, *b*. Single spheroids. *c*. Division of a spheroid, commencing by the separation of the fibres. *d*. a large coil of fibres in the very act of division. *e*. Fibres separated from one another by the breaking up of the gelatinous envelope. *f*. Fibres in ring-shaped pieces. × 600.

FIG. 3.—*Streptothrix Foersteri;* branched, and screw-form fibres from a tallowy mass, taken from the lachrymal canal of a human subject. *a*. Fibres with which are embedded *Micrococci;* the rest were isolated by the separation of the Micrococci. *b*. A thicker mycelium-like fibre. × 600.

FIG. 4.—*Cladothrix dichotoma*. Dichotomous fibres forming white mucous masses on the surface of putrefying fluids. × 600. *a*. The false dichotomy is clear. × 600.

FIG. 5.—*Bacillus anthracis:* Bacilli from the blood of a cow that had died of splenic fever, examined after death. × 600.

FIG. 6.—*Bacillus subtilis*. *a*. Bacterian fibres from an extract of rennet, in lively movement; *b*, with spores; *d*, spores with short fibres (? budding spores).

FIG. 7.—*B. subtilis*. Bacterian fibres from a solution of boiled peas in which butyric fermentation had begun; the spores in a row. × 600.

FIG. 8.—*Micrococci* with embedded Bacillus-spores.

FIG. 9.—*Micrococcus Bombycis:* from the gastric fluid of a living silkworm, affected with the disease (flaccidezza); oval cells arranged in rows, either in pairs or in chains of varying length.

FIG. 10.—*Clathrocytis roseo-persicina:* a net-like cell family.

FIG. 11.—*Monas Warmingii:* motile Monads with flagellum: in * the division is just beginning, in ** it has gone farther. × 600.

FIG. 12.—*Monas Okenii:* actively motile monads; and division beginning. × 600.

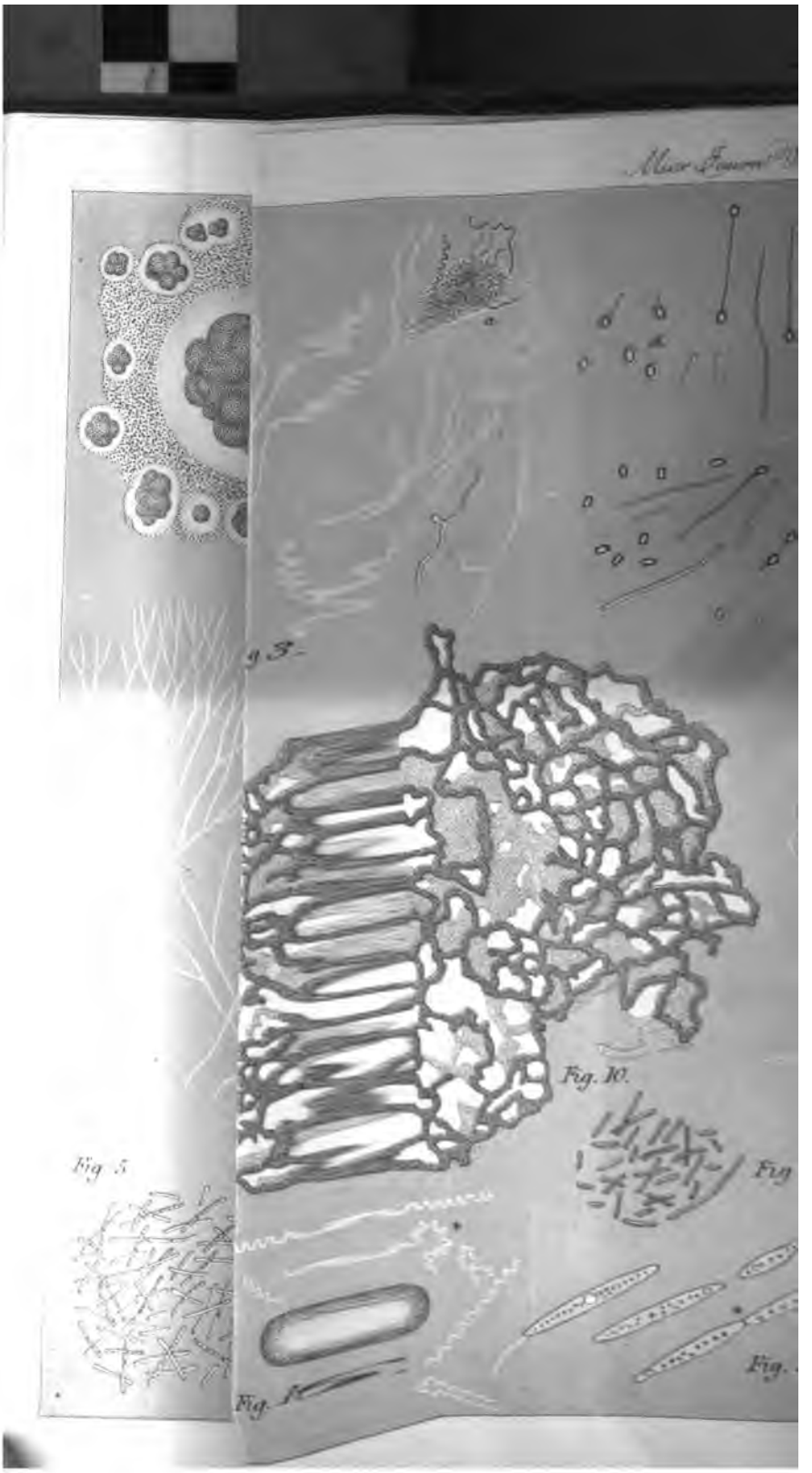


Fig. 10.
Fig. 5

EXPLANATION OF PLATE XX—*Continued.*

FIG. 13.—*Monas vinosa :* Monads in lively movement and in divisi flagella were not clearly seen. × 600.

FIG. 14.—*Rhabdomonas rosea ;* slowly moving monads; * in the division. × 600.

FIG. 15.—*Ophidomonas* (*Spirillum*) *sanguinea ;* Monads with flage one, at both (*) ends: ** semilunar form. × 600.

FIG. 16.—*Spirochæte Obermeieri ;* screw-fibres, actively moving blood-corpuscles (**); * shortly before the cessation of the fever.

FIG. 17.—*Bacillus ruber*: rods, forming red spots on rice.

FIG. 18.—*Micrococcus fulvus :* colonies, forming rust-red drops of on horse-dung.

All the figures were drawn with Hartnack's objectives. Figs. 10 Kirchner, 16 by Dr. Weigert, and the rest by Cohn.

The explanation of Plate XXI, illustrating Mr. Ar Resumé of Recent Contributions to our Knowledg "Freshwater Rhizopoda," will be given with the number.

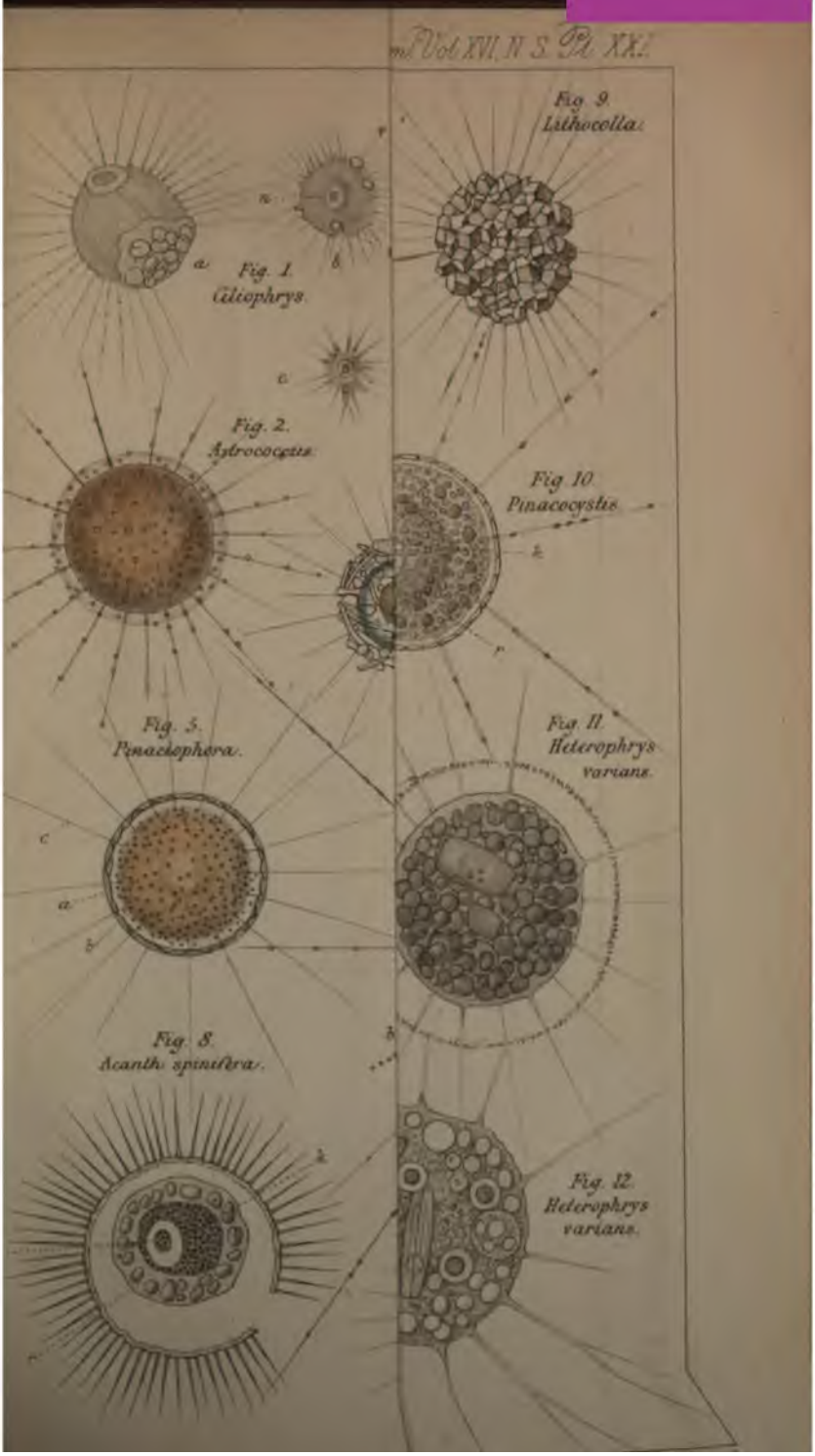


Fig. 1.
Ciliophrys
Fig. 2.
Astrococcus
Fig. 3.
Pinaciophora.
Fig. 8.
Acanth. spinifera.
Fig. 9
Lithocolla.
Fig 10
Pinacocystis
Fig. 11.
Heterophrys
varians.
Fig. 12.
Heterophrys
varians.

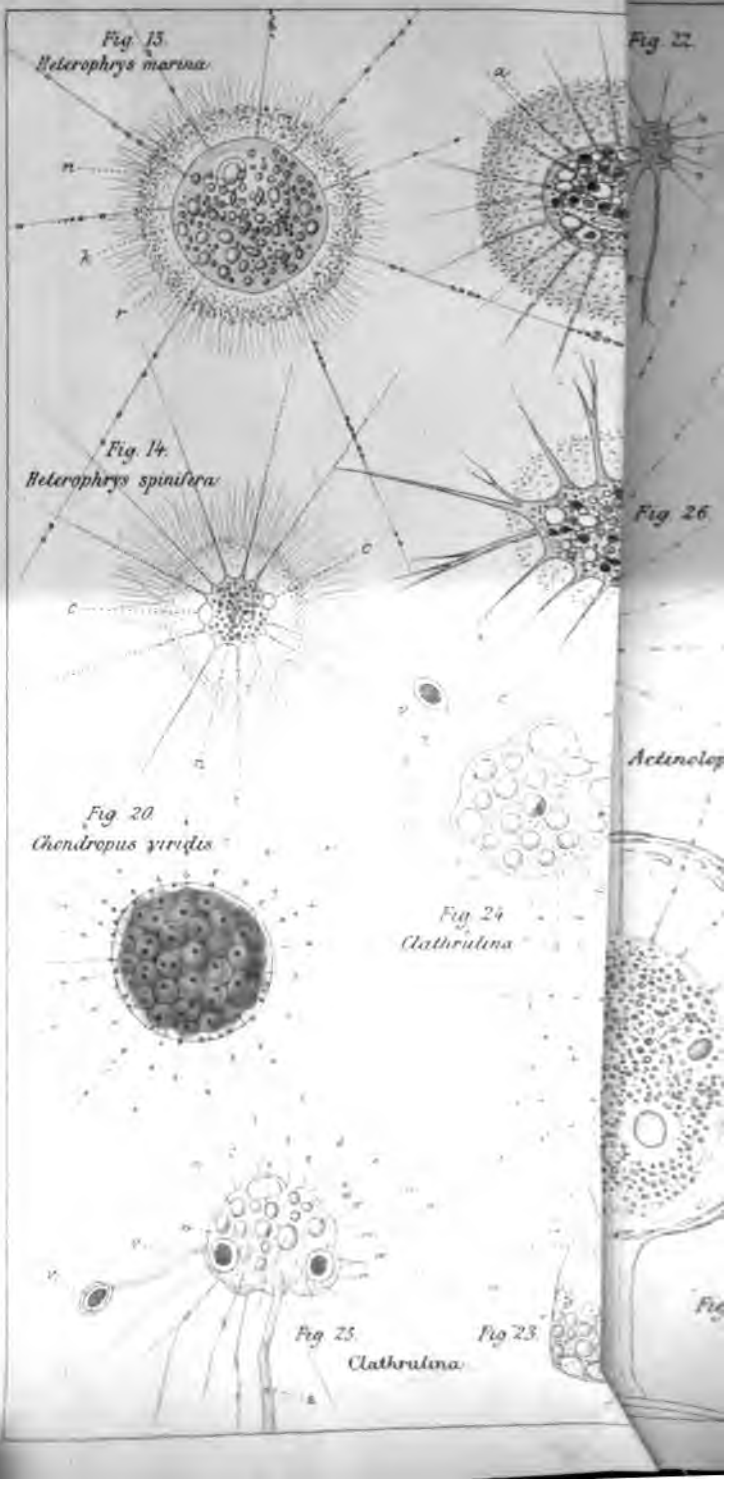


Fig. 13.
Heterophrys marina
Fig. 14.
Heterophrys spinifera
Fig. 20
Chondropus viridis
Fig. 24
Clathrulina
Fig. 25.
Clathrulina
Fig 23.
Fig 22.
Fig. 26
Actinolop

EXPLANATION OF PLATES XXI & XXII,

Illustrating Mr. Archer's Résumé of Recent Contributions to our Knowledge of "Freshwater Rhizopoda."

PLATE XXI.

FIG. 1.—*Ciliophrys infusionum*, Cienk.; *a*, *b*, *c*, *d*, in the motionless state (*n*, nucleus with nucleolus; *v*, contractile vacuoles); *e*, *f*, *g*, *h*, zoospores, *f* still retaining some pseudopodia (*n*, nucl.; *v*, vacuoles). × 700 (?)

FIG. 2.—*Astrococcus rufus*, Greeff. × 700.

FIG. 3.—*Astrodisculus ruber*, Greeff. × 600. (The colouring of the central body and of the round granules external to it has not been rendered of a sufficiently highly ruby-red colour to be quite in accordance with Greeff's original figure.)

FIG. 4.—*Elaeorhanis cincta*, Greeff. × 600.

FIG. 5.—*Pinaciophora fluviatilis*, Greeff; *a*, outer siliceous 'skeleton;' *b*, inner body-mass; *c*, pseudopodia. About × 400.

FIG. 6.—*Acanthocystis aculeata*, Hertwig et Lesser; *k*, endosarc; *r*, ectosarc; *c*, *c*, contractile vacuoles; *b*, a pseudopodium contracted (owing to some shock); the figure is drawn, as it were, through an optical section of the body; the nucleus, mostly rendered evident by acetic acid, has been left out of the drawing because in the example the authors sketched, as is the case mostly in the fresh state, it was not visible. (The amplification not stated, beyond that the figure was drawn under "Zeiss. F. Oc. III.")

FIG. 7.—*Acanthocystis flava*, Greeff. (× 400 ?)

FIG. 8.—*Acanthocystis spinifera*, Greeff; *k*, endosarc; *r*, ectosarc; *n*, nucleus, after treatment with acetic acid; after Hertwig and Lesser. (Drawn under same amplification as Fig. 6.)

FIG. 9.—*Lithocolla globosa*, Eilhard Schulze. × 600.

FIG. 10.—*Pinacocystis rubicunda*, Hertwig et Lesser; *k*, endosarc; *r*, ectosarc; *n*, nucleus. (Drawn under same amplification as Fig. 6.)

FIG. 11.—*Heterophrys varians*, Eilh. Schulze. An example surrounded by the gelatinous envelope with four dark granules and some food particles. × 400.

(This is the same rhizopod as that called *Heliophrys variabilis* by Greeff, see figs. 15—18; inasmuch as it is not a homaxial form, it is not a Heliozoan, therefore it should not be relegated to Heterophrys; hence Greeff's name, so very nearly like, indeed, to that of Schulze, should stand.)

FIG. 12.—Same, a naked reptant animal, containing four evident nuclei and a number of pulsating vacuoles towards the marginal region. Granules are not present; on the other hand, two diatoms incepted as food. × 600.

PLATE XXII.

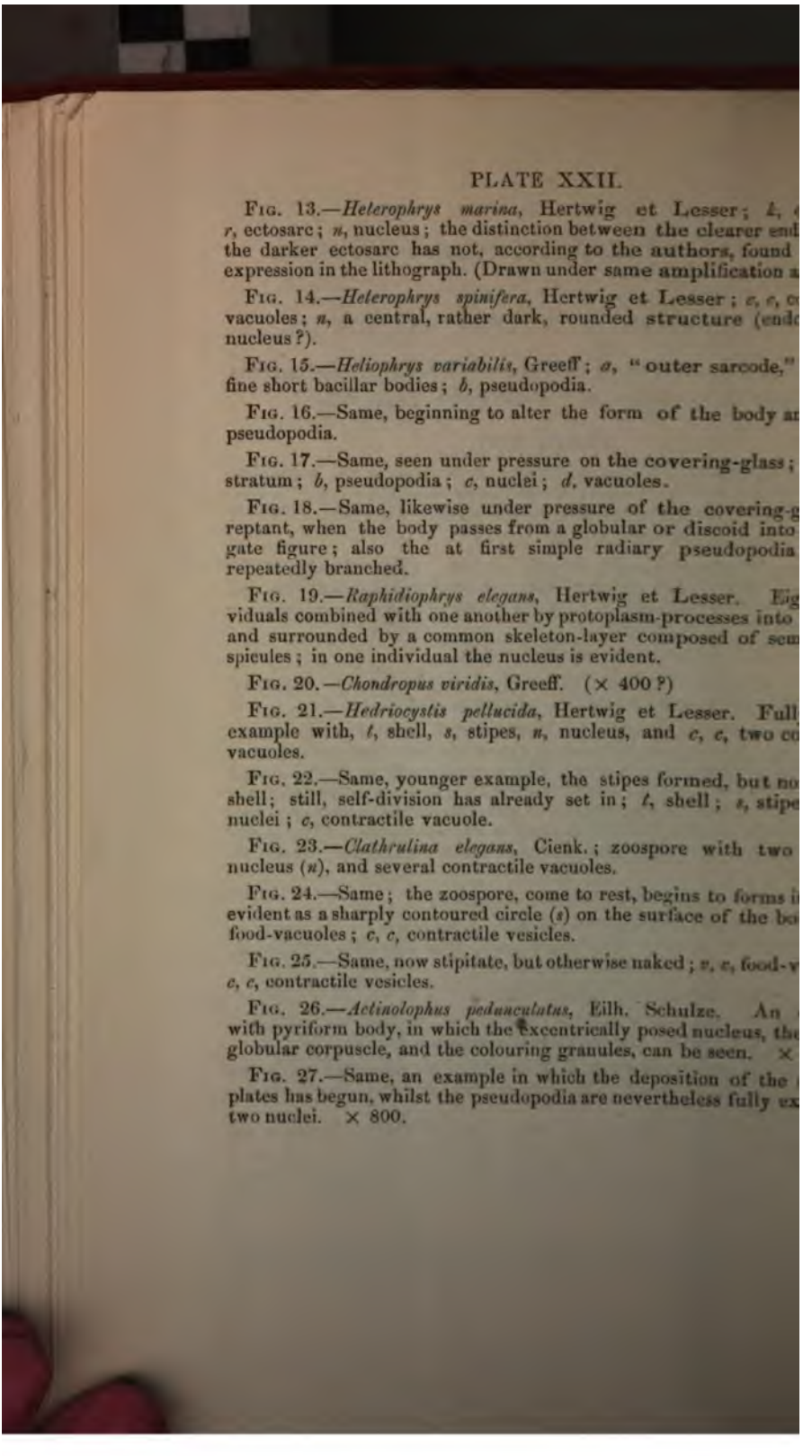


FIG. 13.—*Heterophrys marina*, Hertwig et Lesser; *k*, *r*, ectosarc; *n*, nucleus; the distinction between the clearer end the darker ectosarc has not, according to the authors, found expression in the lithograph. (Drawn under same amplification a

FIG. 14.—*Heterophrys spinifera*, Hertwig et Lesser; *c*, *c*, c vacuoles; *n*, a central, rather dark, rounded structure (endo nucleus ?).

FIG. 15.—*Heliophrys variabilis*, Greeff; *a*, "outer sarcode," fine short bacillar bodies; *b*, pseudopodia.

FIG. 16.—Same, beginning to alter the form of the body a pseudopodia.

FIG. 17.—Same, seen under pressure on the covering-glass; stratum; *b*, pseudopodia; *c*, nuclei; *d*, vacuoles.

FIG. 18.—Same, likewise under pressure of the covering-g reptant, when the body passes from a globular or discoid into gate figure; also the at first simple radiary pseudopodia repeatedly branched.

FIG. 19.—*Raphidiophrys elegans*, Hertwig et Lesser. Fig viduals combined with one another by protoplasm-processes into and surrounded by a common skeleton-layer composed of sca spicules; in one individual the nucleus is evident.

FIG. 20.—*Chondropus viridis*, Greeff. (× 400 ?)

FIG. 21.—*Hedriocystis pellucida*, Hertwig et Lesser. Full example with, *t*, shell, *s*, stipes, *n*, nucleus, and *c*, *c*, two co vacuoles.

FIG. 22.—Same, younger example, the stipes formed, but no shell; still, self-division has already set in; *t*, shell; *s*, stipe nuclei; *c*, contractile vacuole.

FIG. 23.—*Clathrulina elegans*, Cienk.; zoospore with two nucleus (*n*), and several contractile vacuoles.

FIG. 24.—Same; the zoospore, come to rest, begins to forms i evident as a sharply contoured circle (*s*) on the surface of the bo food-vacuoles; *c*, *c*, contractile vesicles.

FIG. 25.—Same, now stipitate, but otherwise naked; *v*, *v*, food-v *c*, *c*, contractile vesicles.

FIG. 26.—*Actinolophus pedunculatus*, Eilh. Schulze. An with pyriform body, in which the excentrically posed nucleus, the globular corpuscle, and the colouring granules, can be seen. ×

FIG. 27.—Same, an example in which the deposition of the plates has begun, whilst the pseudopodia are nevertheless fully ex two nuclei. × 800.

Fig. 1

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

a

b

c

d

e

Fig. 6

a

b

Fig. 7

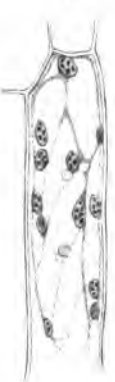


Fig. 8.

Fig. 9.

Fig 10.

M^r Farlow & Erskine Lith^r E

EXPLANATION OF PLATE XXIII,

Illustrating Mr. Francis Darwin's paper on the Process of Aggregation in the Tentacles of *Drosera rotundifolia*.

FIG. 1.—A cell from the pedicel of the tentacle, in a normal unaggregated condition. It is filled with a homogeneous crimson fluid through which protoplasmic currents and yellow chlorophyll bodies are faintly seen.

FIG. 2.—A cell in the aggregated condition produced by a solution of carbonate of ammonia. A large vacuole seen in one of the masses of aggregated matter.

FIG. 3.—A cell which has lately died in a state of aggregation; the flowing protoplasmic network is coagulated and is seen stretching from one chlorophyll body to the next.

FIG. 4.—Part of a cell showing chlorophyll bodies collected into a heap by the action of carbonate of ammonia (part of cell omitted).

FIG. 5.—A cell in an aggregated condition; the cover glass having been pressed; the firm consistency of the aggregated masses in the later stages of aggregation is shown by the way in which they break into starlike forms.

FIG. 6.—A cell in which aggregation was produced by an infusion of raw meat. A, B, C, D, and E, show changes of form successively assumed by the aggregated matter; the changes occurred so rapidly that it was impossible to copy the appearance of the *whole* cell at a given moment.

FIG. 7.—A cell which is undergoing dissolution of the state of aggregation, drawn at successive periods, A being the earlier of the two. It shows the change from a light to a dark colour which occurs, this change travelling *up* the gland from cell to cell and also *up* each individual cell. Simple changes of form are also shown.

FIG. 8.—Shows the streaming network of protoplasm in a pale cell containing chlorophyll bodies. The current is seen to flow from one chlorophyll-body to another.

FIG. 9. }
FIG. 10. } Simpler currents in pale cells.

EXPLANATION OF PLATE XXIV,

Illustrating Professor Lankester's Remarks on the Shell-gland of Cyclas and the Planula of Limnæus.

FIG. 1.—Embryo of *Cyclas cornea*, showing both shell-gland and byssus-gland. *Sh.-Gl.* Shell-gland. *By.-Gl.* Byssus-Gland. *Ph.* Pharynx. *F.* Foot *R. P.* Rectal peduncle.

FIG. 2.—An embryo of *Cyclas cornea* somewhat further advanced in development. The shell-gland has now flattened out, and there is evidence of a delicate secretion on its surface, the single primordial shell. Letters as in fig. 1.

In neither of these figures is any attempt made to give histological detail; but the drawing is intended solely to represent the appearances in a half-diagramatic style which are seen when the embryo is living, and examined with Hartnack's obj. 8, ocular 4.

FIG. 3.—Morula of Limnæus seen from the antiklastic pole. Four larger cells are seen, between which, at the periphery, are the tips of four encroaching smaller cells.

FIG. 4.—Lateral view of a morula of Limnæus of the same age. The large size of the cells at the antiklastic pole is obvious. *D. C.* Directive corpuscles. *Ch.* Chorion.

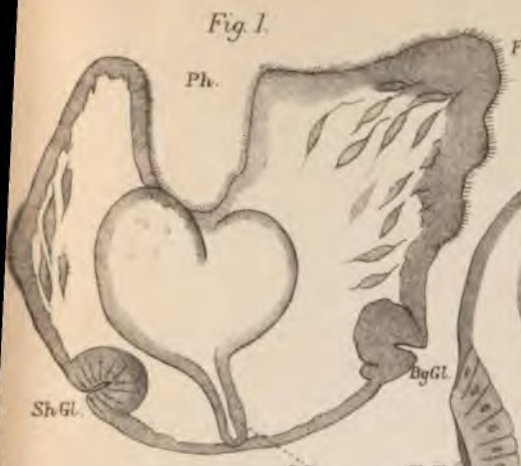
FIG. 5.—A morula of Limnæus, a little more advanced than that of fig. 3, with eight in place of four encroaching cells, viewed from the antiklastic pole.

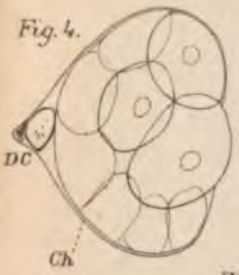
FIG. 6.—A similar morula seen from the side. *D. C.* Directive corpuscles. *Ch.* Chorion.

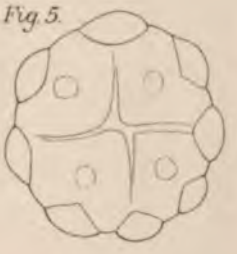
FIG. 7.—Optical section through a morula a little more advanced than that of fig. 6, showing the formation of a cavity at the klastic pole, and the long processes (mesoblastic?) of the pale cells of the klastic hemisphere. *D. C.* Directive corpuscles. *Ch.* Chorion.

FIG. 8.—A surface view of the same embyro.

FIG. 9.—Outline of the invaginate planula or gastrula of Limnæus, to show the elongated character of the blastopore. *Or.* Oral end of the blastopore. *Rect.* Rectal end.







E. R. Lankester, del.

McFarlane & Erskine Lith.

CYCLAS AND LIMNÆUS.

THE NEW YORK
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AND

EXPLANATION OF PLATE XXV,

; Professor Ray Lankester's Memoir "On the ence of the Blastopore and Anus in Paludina "

nsegmented egg as found in the upper part of the oviduct. vesicle is not observable: the protoplasm is charged with in the form of yellow granules. As seen in the living state d light. Hartnack's Oc. iv, Obj. 8.

arly stage of cleavage, showing the hyaline character of the the edges of the large cleavage-spheres and the contained granules. As seen in the living state by transmitted light. c. iv, Obj. 8.

lastula stage. The cells have now further divided, and the es the form of a hollow sphere, the wall of which consists of r of cells. As seen in the living state by transmitted light. c. iv, Obj. 8.

ommencement of the gastrula-invagination. *Bl.* Blastopore. e corpuscle (this is omitted in the previous figures). As seen state, with slight exaggeration of the transparency. Hart-, Obj. 8. *Ect.* Ectoderm or epiblast. *End.* Endoderm or *l.* Blastopore. *Bc.* Cavity of the blastula- or segmentation-

ompletion of the gastrula. The endoderm, *end*, has now applied to the ectoderm, and bounds the primitive stomach or *Bl.* Blastopore. *Blm.* Margin of the blastopore. *Ect.* Ec-. Endoderm. *Dc.* Directive corpuscle. As seen in the living mitted light. Hartnack's Oc. iv, Obj. 8.

iagram of median vertical section of the specimen drawn in Blastopore. *Ae.* Archenteron. *Dc.* Directive corpuscle.

rochosphere stage. A circlet of cilia now divides the embryo anterior and posterior regions. A space, the mesoblastic m traversed by delicate filaments connecting endoderm and ow apparent. The blastopore is still widely open. *Dv.* Dome area. *Vr.* Ridge or ciliated margin of the velum area.

en is viewed from the pedal or ventral surface; that on which and anus subsequently lie. Seen by transmitted light with . iv, Obj. 8.

edian vertical dorso-ventral section of a similar embryo as ing specimen, the transparency of the parts being slightly *Dv.* Dome of the velum area. *Vr.* Ridge of the velum. e. *F.* First indication of the foot. *Sm.* Position *subsequently*

occupied by the mouth. *Ect.* Ectoderm. *End.* Endoderm. *Mes.* Me derm.

FIG. 9.—Lateral surface view of a somewhat older specimen. Lett as in Fig. 8.

FIG. 10.—A specimen still further advanced in development, seen fr the pallial or dorsal surface, so as to show the shell-gland. The sup ficial cells of this region are drawn as they are brought to view by action of dilute acetic acid. *Dv.* Dome of the velum area. *Vr.* Ridge the velum.

FIG. 11.—The same specimen before treatment with acetic acid, viev from the pedal or ventral surface by transmitted light with Hartnack's iv, Obj. 8. *Vr.* Ridge of the velum. *Sm.* Site *subsequently* occupied the mouth. *Bl.* Blastopore. *Ae.* Archenteron. *Ect.* Ectoderm. *E* Endoderm. *Mes.* Mesoderm. *Pi.* Pedicle of invagination or rec peduncle.

FIG. 12.—Lateral view of the same specimen, similarly viewed as transparent object. *Dv.* Dome of the velum area. *Vr.* Marginal ridge the velum. *Sm.* Site *subsequently* occupied by the mouth or stomod invagination. *F.* Foot. *Bl.* Blastopore. *Shgl.* Shell-gland. *Pi.* Pedi of invagination.

FIG. 13.—Lateral superficial view of a specimen further advanced size and development, representing the Veliger stage. *Dv.* Dome of velum area. *Vr.* Marginal ridge of the velum. *M.* Mouth or orifice the stomodæal invagination. *F.* Foot. *An.* Anus coinciding with blastopore. *Shgl.* Shell-gland.

FIG. 14. The same specimen seen as a transparent object in med dorso-ventral vertical section. *Ect.* Ectoderm. *Mes.* Mesoderm. *E* Endoderm. *Ae.* Archenteron. *Ph.* Stomodæum, the so-called phary being the invagination of the ectoderm to form the most anterior port of the alimentary canal. Other letters as in the preceding figure.

FIG. 15.—Surface view from the anterior pole of an embryo intermedi in age between that represented in Fig. 12 and that represented in Fig. The embryo sometimes appears in this position when swimming. Comp this Journal, Vol. XIV, New Series, Pl. XVII, fig. 6, for a similar view the Veliger of Limnæus.

FIG. 16.—The same embryo as that of Figs. 13 and 14, seen from pedal or ventral surface. *Dv.* Dome of the velum area. *Vr.* Margi ridge of the velum. *M.* Mouth.

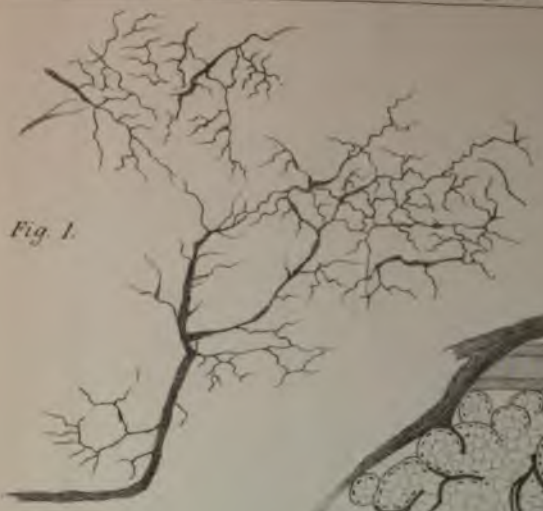

Fig. 1.

Fig. 2.

Fig. 3.

Fig.

Fig

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XPLANATION OF PLATE XXVI,

ing Mr. P. Kidd's "Note on the Lymphatics of Mucous Glands."

Lymphatics of pharynx viewed from the surface under a lens.

Injected lymphatics of mucous glands of pharynx.

Lymphatic spaces around lobules of mucous gland of pharynx, ened specimen.

EXPLANATION OF PLATE XXVII,

ng Mr. Vines' paper on "Recent Views as to the sition of the Fibro-vascular Bundles of Plants."

r Schwendener):

erse section of a fibro-vascular bundle of the stem of Juncus ulatus. *f.* Bast-fibres. *b. s.* Bundle-sheath. *p.* Phloëm. ylem.

r Dippel):

erse section of bundle of stem of Ranunculus bulbosus. Ground tissue. *f.* Bast-fibres, the inner layer of which forms incomplete bundle-sheath (Steifungs-scheide). *x.* Xylem. Xylem vessels. *p.* Phloëm. *p. v.* Phloëm vessels. Phloëm parenchyma.

r Dippel):

erse section of phloëm of Juniperus communis. *f.* Bast- s. *v.* Phloëm vessels. *p.* Phloëm parenchyma.

r Dippel):

erse section of bundle from the leaf of Gladiolus segetum. ast-fibres. *p.* Phloëm. *x.* Xylem.

r Dippel):

erse section of an external fibro-vascular bundle of Dracæna o. *f.* The pitted cells. *v.* Phloëm vessels. *p.* Phloëm nchyma.

r Dippel):

erse section of bundle from stem of Bryonia alba. f^1. Peri- al, f^2. Central bast-fibres. p^1. Peripheral, p^2. Central phloëm. ylem.

EXPLANATION OF PLATE XXVIII,

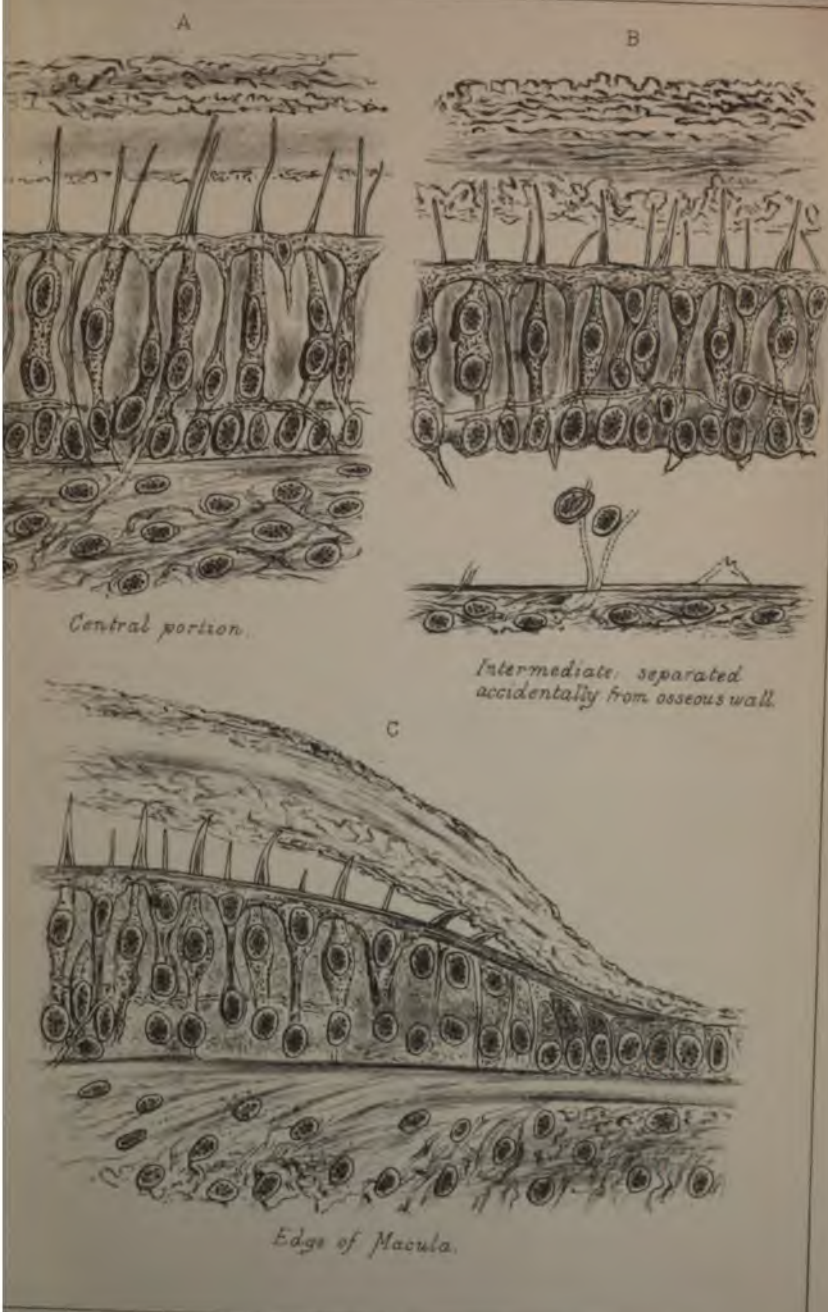
Illustrating Dr. Pritchard's paper "On the Termination of the Nerves in the Vestibule and Semicircular Canals of Mammals."

Portions of a transverse section through the macula acustica of saccule of cat, ten months old. × 750 dia.

A. Centre of macula. Otolith mass above, into which the long cilia are projecting; the otoliths themselves having been dissolved out. Nerve-epithelium, lying on the modified periosteum, consists of thorn-cells and atrophied bristle-cells kept in position by the membrana reticularis.

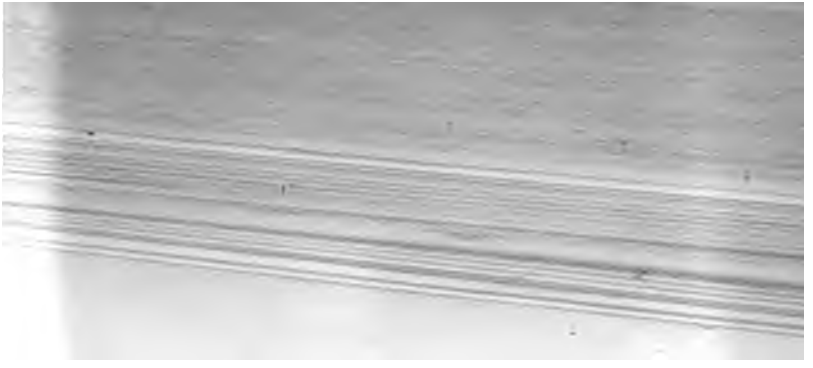
B. Intermediate portion. Otolith mass as before, cilia not so long. Thorn-cells as before; bristle-cells perfect towards the outer side, atrophying towards the inner. Nerve-epithelium has been separated by accident from tissue beneath, whence nerve-filaments are seen passing upwards.

C. Edge of macula. Otolith mass thinning off towards the columnar epithelium, cilia short and stumpy. Membrana reticularis becoming gradually lost. The nerve-epithelium changing by degrees into ordinary columnar cells.



Richard. F.R.Miller, del. Mt Packham & Erskine Lith.rs Edin.

SECTION THROUGH MACULA ACUSTICA OF SACCULE OF CAT.
X 360 Diameters.



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